Cancer
For researcher Series

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Dedication to my parents and my love for their support of me.

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With special thanks to my family and all those who helped me in this way

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This text is dedicated to the dear ones, my mother and father.

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Chapter 1: History

The physicians of ancient Egypt treated patients for several forms of cancer by mixing medicine and religion; prescribing pills and pigs' ears; and dispensing ointments, enemas, castor oil, suppositories, poultices, and parts of animals. Cancer, as an illness of man, has been described in the history of medicine since the earliest medical records were kept.

After the decline of Egypt, the next chapters of medical and scientific history were written in Greece and Rome. The origin of the word cancer is credited to the Greek physician Hippocrates (460-370 BC), considered the "Father of Medicine." Lack of anesthesia and antiseptic conditions made surgery a risky choice for cancer treatment in the 17th century. The most significant achievement in medicine in the 17th century was William Harvey's proof of the continuous circulation of the blood in a contained system. In 1761, Giovanni Morgagni was the first to perform autopsies to relate the patients' illness to the pathologic findings after death. This laid the foundation for scientific oncology, the study of cancer. The birth of scientific oncology came in the 19th century with Rudolf Virchow's discovery and use of the modern microscope. As Morgagni had correlated the autopsy findings with the clinical course of illness, so Virchow correlated the microscopic pathology. When anesthesia became available in 1846, there emerged the great surgeons whose work so rapidly advanced the art that the next hundred years became known as "the century of the surgeon." William Stewart Halsted, professor of surgery at Johns Hopkins University, developed the radical mastectomy during the last decade of the 19th century. As the 19th century was drawing to a close, in 1896 a German physics professor, Wilhelm Conrad Roentgen, presented a remarkable lecture entitled "Concerning a New Kind of Ray." Roentgen called it the "X-ray", with "X" being the algebraic symbol for an unknown quantity. Within months, systems were being devised to use x-rays for diagnosis, and within 3 years radiation was used in the treatment of cancer. Over the years, the development and use of chemotherapy drugs has resulted in the successful treatment of many people with cancer. The first cure of metastatic cancer was obtained at the National Cancer Institute in 1956 when methotrexate was used to treat choriocarcinoma. Other cancers that can now be cured regularly with chemotherapy, even when widespread. Responding to public

pressure and a concerted campaign by the American Society for the Control of Cancer, in 1937, Congress made the conquest of cancer a national goal with a unanimous vote to pass the National Cancer Institute Act. Franklin D. Roosevelt signed this act on August 5, 1937. Advances in computer technology in the 1980s transformed diagnostic imaging, making it possible to visualize organs and soft tissues in a detail that had previously been available only with anatomic dissections. Imaging with computed tomography, positron-emission tomography, magnetic resonance imaging, and ultrasound provided ways of detecting tumors in areas not accessible with a physical examination or x-rays alone. Another technique, immunodiagnostics, used antibodies, linked to radioactive isotopes, to seek out and identify cancerous growths. Epidemiological research indicates that a third of cancer deaths are linked to diet, and study after study proves that regular exercise offers protection from some kinds of cancer. Cancer survivors deal with unique issues such as long-term physical, psychological, and social side effects of treatment; the economic costs of medical care and recovery; and the possibility of future malignancies. Imaging plays an important role in the detection, diagnosis, treatment, and monitoring of many different types of cancer.

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Chapter 2: Cancer biology

The cell is humankind's most basic unit. It is also the lightest unit in the body, able to handle out all of life's functions. Every one of the internal tissues are made up of unique cells that carry out duties like as oxygen transportation, nutrition digestion, waste outflow, motility, reproduction, and understanding.

To ensure that each organ functions properly, aged or damaged cells should be changed, and certain cells should grow in reaction to environmental changes. such as rapidly creation of some white blood cells in an infection. Furthermore, the liver and hormonal tissues regularly regenerate injured cells in response to injury.

Cell division is the process of cell reproduction. Natural cells division is a precisely calibrated mechanism. The DNA of a cell determines its development, reproduction, and restriction.

DNA is a complicated structure produced in the cell nucleus. The pattern for everything a cell performs is DNA. In a human cell, DNA is organized into 46 different sections called chromosomes, thus with more than 100,000 genes (sorted in couples, 23 chromosomes from each biological parent). A gene is a piece of DNA that defines the structure of a protein by occupying a certain place on a chromosome. Each gene leads a cell to produce a specific protein, which is required for growth and development as well as completing out critical biochemical processes in the body, based on number of biochemical stages.

Cells only divide once they get the correct signals from growth factors in the bloodstream or from a cell they come into direct contact with. When a person loses blood, erythropoietin (a growth factor) generated in the kidneys, flows in the bloodstream and informs the bone marrow to create additional blood cells.

When a cell receives the order to split, it moves through the cell cycle, which consists of various stages that must be fulfilled before the division may be completed. Checkpoints at each stage of the process ensure that everything is running well.

Cell multiplication entails a number of steps, all of which must be completed appropriately in order for a cell to divide successfully. A cell can become cancerous if something fails throughout this sophisticated cycle.

A cancer cell is one that multiplies uncontrollably. Cancer cells, apart from natural cells, reject messages to halt multiplying, specialize, or death and discharge. Cancer cells that develop uncontrollably and unable to identify their original natural boundaries may expand to parts of the body that they do not originate.

Several genes in a cancer cell mutate, leading the cell to become dysfunctional. Gene mutations are classified into two categories. A dominating mutation is formed by a defect in one of two genes in a pair. A mutant gene, for example, might generate a faulty protein that keeps the growth-factor receptor on a cell's surface "active" even though no growth factor is available. As a result, the cell is constantly being told to multiply. An oncogene is a term used to describe a dominant "gain of function gene."

The other form of mutation is recessive mutation, which occurs when both genes in a pair are disrupted. A natural gene called p53, creates a protein which "switches off" the cell cycle. The p53 gene's principal purpose is to inhibit future malignant cells by repairing or destroying faulty cells. A tumor suppressor gene is one of these genes. Even if one of the p53 genes in a pair is damaged, the other gene still can manage the cell cycle. The "off" function is gone when both genes are defective, and cell cycle isn't any more under control.

Once active oncogenes are generated or tumor suppressor genes are deleted, irregular cell division might result. actuality,

multiple mutations are required for a cell to become cancerous. Both sorts of mutations might happen in some instances.

Viruses can also induce irregular cell division. Since the cell carries a cancer-producing virus, the genomes could be fine, however the protein might not always run correctly.

The way a cancer cell acts is determined by which mechanisms aren't operating normally. Certain cancerous cells simply proliferate and generate new cancer cells, resulting in a tumor tissue that remains in which it started. Some cancerous cells can infiltrate healthy tissue, enter the bloodstream, and spread to other parts of the body.

To summarize, cancerous cells have flaws in normal cellular operations that let them to proliferate, infiltrate neighboring cells, and move through the vascular and lymphatic systems. Genetic mutations, which are occasionally generated by infectious viruses, are the source of these disorders.

2.1 cellular and molecular

For many patients with cancer of different cell types a number of prognostic factors (e.g. age, stage of disease, etc.) have been clearly defined. However, it is also apparent that other factors may be important as considerable heterogeneity exists among patients in response to cy; totoxic therapies. Currently available information suggests that biological properties inherent within the mmour cells themselves may be of prognostic importance. These properties, including the expression of the drug or radiation resistant genes, or oncogenes coding for more malignant behaviour, may account for observed differences in responses to cytogenic therapy and survival. Recent

developments in molecular biology have allowed the characterisation of molecular events involved in the pathogenesis of many cancers. The technological advances have led to the discovery of a series of genetic changes in cancer cells including point mutations; chromosomal rearrangements or deletions; gene amplifications and altered gene expression. For many cancers specific oncogenes have been identified which are associated with a more aggressive growth behaviour both in vitro and in vivo; resistance to cytotoxic therapy and shortened survival. Thus the identification of specific genetic abnormalities for individual tumours will aid the diagnosis and in defining the prognosis of patients with specific cancers 1-z. One of the earliest reports of the clinical relevance of oncogene amplification in human tumours was the report by Schwab and colleagues who showed that patients with neuroblastoma, but whose tumours were amplified for the N-myc oncogene, had a significantly worse survival than patients whose tumours lacked this amplification s. More recently, as reported elsewhere in this Journal, this work has been extended to evaluate N-myc oncogene amplification in a range of paediatric tumours. The study of McQuaid and O'Meara conf'mns the importance of this oncogene in the biology of neuroblastoma 4. It is clear however that many oncogenes exist which function at different cellular levels, e.g., DNA; code for growth factors or their receptors etc., and it is likely that for many tumours a complex interaction of many genes may be involved in the growth and spread of cancers. The study of the role of oncogenes allows a better understanding of the growth control mechanisms within a cell. The clarification of these events in addition to aiding diagnosis and prognosis, may also allow the design of more specific therapies in the treatment of these diseases. Molecular and biological studies have also greatly increased our understanding of drug resistance. Modem chemotherapy regimens have dramatically changed the prognosis of patients with a variety of cancers including Hodgkin's disease, lymphomas, acute leukaemias and several solid tumours including testicular,

ovarian, small cell lung cancer and many paediatric cancers including primary bone tamours. For these tumours, a cure is a potential and realistic goal. Unfortunately when a relapse occurs, further responses to chemothterapy are uncommon due to the development of drugresistance. For other less responsive tamours, e.g., colon or gastric cancers, drug resistance may be present at the time of diagnosis of the tumour. The development of drug resistance occurs primarily at a cellular level as a consequence of genetic instability. Genetic alterations such as gene amplification and translocation can lead to altered gene products such as target enzymes or membrane proteins which may directly lead to the development of drug resistance. One type of drug resistance which develops following exposure repeatedly to natural cytotoxic agents such as vincristine is known as multidrug resistance (MDR). In MDR malignant cells become resistant to many drugs that show little structural similarity or mode of action. Thus such turnouts that exhibit the MDR phenotype are likely to be resistant to most combination chemotherapeutic regimens. Recently it has been discovered that this phenotype is associated with the expression of an integral membrane protein of 170 Kd - the p-glycoprotein. The mechanism of resistance associated with p-gly is energy dependent and decreases drug accumulation in tumour cells by enhancing drug efflux from cells, resulting in a net decrease of intracellular concentration of drug in resistant cells. The recognition of this type of drug resistance has major implications in medical oncology: 1) Tumour cells can be"typed" by in situ hybridisation or immunohistochemistry for this protein therefore allowing for more selective chemotherapeutic regimens. 2) Understanding this form of drug resistance will allow the development of agents specifically designed to circumvent its effect (e.g. calcium channel-blocking agents). While it is likely that several different pathways may allow for the development of drug resistance, the characterization of these mechanisms, and their evaluation in clinical trials, may greatly help in our treatment of drug resistant turnouts.

2.2 Genetics

cancer is responsible for one in eight deaths worldwide. It encompasses more than 100 distinct diseases with diverse risk factors and epidemiology which originate from most of the cell types and organs of the human body and which are characterized by relatively unrestrained proliferation of cells that can invade beyond normal tissue boundaries and metastasize to distant organs. Early insights into the central role of the genome in cancer development emerged in the late nineteenth and early twentieth centuries from studies by David von Hansemann and Theodor Boveri. Examining dividing cancer cells under the microscope, they observed the presence of bizarre chromosomal aberrations. This led to the proposal that cancers are abnormal clones of cells characterized by and caused by abnormalities of hereditary material. Following the discovery of DNA as the molecular substrate of inheritance and determination of its structure, this speculation was supported by the demonstration that agents that damage DNA and generate mutations also cause cancer. Subsequently, increasingly refined analyses of cancer cell chromosomes showed that specific and recurrent genomic abnormalities, such as the translocation between chromosomes 9 and 22 in chronic myeloid leukaemia (known as the 'Philadelphia' translocation), are associated with particular cancer types. Finally, it was demonstrated that introduction of total genomic DNA from human cancers into phenotypically normal NIH3T3 cells could convert them into cancer cells. Isolation of the specific DNA segment responsible for this transforming activity led to the identification of the first naturally occurring, human cancer-causing sequence change—the single base G . T substitution that causes a glycine to valine substitution in codon 12 of the HRAS gene. This seminal discovery in 1982 inaugurated an era of vigorous searching for the abnormal genes underlying the development of human cancer that continues today. Here we review the principles of our current understanding of cancer genomes. We look forward to the

explosion of information about cancer genomes that is imminent and the insights into the process of oncogenesis that this promises to generate

Cancer is an evolutionary process

All cancers are thought to share a common pathogenesis. Each is the outcome of a process of Darwinian evolution occurring among cell populations within the microenvironments provided by the tissues of a multicellular organism. Analogous to Darwinian evolution occurring in the origins of species, cancer development is based on two constituent processes, the continuous acquisition of heritable genetic variation in individual cells by more-or-less random mutation and natural selection acting on the resultant phenotypic diversity. The selection may weed out cells that have acquired deleterious mutations or it may foster cells carrying alterations that confer the capability to proliferate and survive more effectively than their neighbours. Within an adult human there are probably thousands of minor winners of this ongoing competition, most of which have limited abnormal growth potential and are invisible or manifest as common benign growths such as skin moles. Occasionally, however, a single cell acquires a set of sufficiently advantageous mutations that allows it to proliferate autonomously, invade tissues and metastasize.

The catalogue of somatic mutations in a cancer genome

Like all the cells that constitute the human body, a cancer cell is a direct descendant, through a lineage of mitotic cell divisions, of the fertilized eggfrom which the cancer patient developed and therefore carries a copy of its diploid genome. However, the DNA sequence of a cancer cell genome, and indeed of most normal cell genomes, has acquired a set of differences from its progenitor fertilized egg. These are collectively termed somaticmutations to distinguish themfrom germlinemutations

that are inherited from parents and transmitted to offspring. The somatic mutations in a cancer cell genome may encompass several distinct classes of DNA sequence change. These include substitutions of one base by another; insertions or deletions of small or large segments of DNA; rearrangements, in which DNA has been broken and then rejoined to a DNA segment from elsewhere in the genome; copy number increases from the two copies present in the normal diploid genome, sometimes to several hundred copies (known as gene amplification); and copy number reductions that may result in complete absence of a DNA sequence from the cancer genome . In addition, the cancer cell may have acquired, from exogenous sources, completely new DNA sequences, notably those of viruses such as human papilloma virus, Epstein Barr virus, hepatitis B virus, human T lymphotropic virus 1 and human herpes virus 8, each of which is known to contribute to the genesis of one or more type of cancer. Compared to the fertilized egg, the cancer genome will also have acquired epigenetic changes which alter chromatin structure and gene expression, and which manifest at DNA sequence level by changes in the methylation status of some cytosine residues. Epigenetic changes can be subject to the same Darwinian natural selection as genetic events, provided that there is epigenetic variation in the population of competing cells, that the epigenetic changes are stably heritable from the mother to the daughter cell and that they generate phenotypic effects for selection to act on. Finally, it should not be forgotten that another genome is harboured within the cancer cell. The thousands of mitochondria present each carry a circular genome of approximately 17 kilobases. Somatic mutations in mitochondrial genomes have been reported in many human cancers, although their role in the development of the disease is not clear.

Acquisition of somatic mutations in cancer genomes

The mutations found in a cancer cell genome have accumulated over the lifetime of the cancer patient. Some were acquired when ancestors of the cancer cell were biologically normal, showing no phenotypic characteristics of a cancer cell. DNA in normal cells

is continuously damaged by mutagens of both internal and external origins. Most of this damage is repaired. However, a small fraction may be converted into fixed mutations and DNA replication itself has a low intrinsic error rate. Our understanding of somatic mutation rates in normal human cells is still relatively rudimentary. However, it is likely that the mutation rates of each of the various structural classes of somatic mutation differ and that there are differences among cell types too. Mutation rates increase in the presence of substantial exogenous mutagenic exposures, for example tobacco smoke carcinogens, naturally occurring chemicals such as aflatoxins, which are produced by fungi, or various forms of radiation including ultraviolet light. These exposures are associated with increased rates of lung, liver and skin cancer, respectively, and somatic mutations within such cancers often exhibit the distinctive mutational signatures known to be associated with the mutagen. The rates of the different classes of somatic mutation are also increased in several rare inherited diseases, for example Fanconi anaemia, ataxia telangiectasia, mosaic variegated aneuploidy and xeroderma pigmentosum, each of which is also associated with increased risks of cancer. The rest of the somatic mutations in a cancer cell genome have been acquired during the segment of the cell lineage in which predecessors of the cancer cell already show phenotypic evidence of neoplastic change. Whether the somatic mutation rate is always higher during this part of the lineage is controversial. For some cancers this is clearly the case. For example, colorectal and endometrial cancers with defective DNA mismatch repair due to abnormalities in genes such as MLH1 and MSH2, exhibit increased rates of acquisition of single nucleotide changes and small insertions/deletions at polynucleotide tracts. Other classes of such ‘mutator phenotypes’ may exist, for example leading to abnormalities in chromosome number or increased rates of genomic rearrangement, although these are generally less well characterized. The merit of an increased somatic mutation rate with respect to the development of cancer is that it increases the DNA sequence diversity on which selection can act. However, it has been suggested that the mutation rates of normal cells may be sufficient to account for the development of some cancers,

without the requirement for a mutator phenotype. The course of mutation acquisition need not be smooth and predecessors of the cancer cell may suddenly acquire a large number of mutations. This is sometimes termed ‘crisis’, and can occur after attrition of the telomeres that normally cap the ends of chromosomes, with the cell having to substantially reorganize its genome to survive. Although complex and potentially cryptic to decipher, the catalogue of somatic mutations present in a cancer cell therefore represents a cumulative archaeological record of all the mutational processes the cancer cell has experienced throughout the lifetime of the patient. It provides a rich, and predominantly unmined, source of information for cancer epidemiologists and biologists with which to interrogate the development of individual tumours.

Driver and passenger mutations

Each somatic mutation in a cancer cell genome, whatever its structural nature, may be classified according to its consequences for cancer development. ‘Driver’ mutations confer growth advantage on the cells carrying them and have been positively selected during the evolution of the cancer. They reside, by definition, in the subset of genes known as ‘cancer genes’. The remainder of mutations are ‘passengers’ that do not confer growth advantage, but happened to be present in an ancestor of the cancer cell when it acquired one of its drivers. The number of driver mutations, and hence the number of abnormal cancer genes, in an individual cancer is a central conceptual parameter of cancer development, but is not well established. It is highly likely that most cancers carry more than one driver and that the number varies between cancer types. On the basis of age–incidence statistics it has been suggested that common adult epithelial cancers such as breast, colorectal and prostate require 5–7 rate-limiting events, possibly equating to drivers, whereas cancers of the haematological system may require fewer. These estimates are supported by experimental studies which show that engineering changes in the functions of at least five or six genes in normal primary human cells is

necessary to convert them into cancer cells. However, recent analyses of somatic mutation data from cancers indicate that the number of drivers might be much higher. Ultimately, direct estimates of the number of drivers in individual cancers will be provided by identifying all the cancer genes and systematically measuring the prevalence of mutations in them. One important subclass of driver is a mutation that confers resistance to cancer therapy. These are typically found in recurrences of cancers that have initially responded to treatment but that are now resistant. Resistance mutations often confer limited growth advantage on the cancer cell in the absence of therapy. Some seem to predate initiation of treatment, existing as passengers in minor subclones of the cancer cell population until the selective environment is changed by the initiation of therapy. The passenger is then converted into a driver and the resistant subclone preferentially expands, manifesting as the recurrence.

The repertoire of somatically mutated cancer genes

The identification of driver mutations and the cancer genes that they alter has been a central aim of cancer research for more than a quarter of a century. It has been a remarkably successful endeavour, with at least 350 (1.6%) of the ,22,000 protein-coding genes in the human genome reported to show recurrent somatic mutations in cancer with strong evidence that these contribute to cancer development. Most were identified by first establishing their physical location in the genome through lowresolution genome-wide screens, in particular cytogenetics for chromosomal translocations in leukaemias and lymphomas. A few were discovered using biological assaysfor transforming activity of whole cancer cell DNA and others through targeted mutational screens guided by biologically well-informed guesswork. Mutations in ,10% of these genes are also found in the germ line, where they confer an increased risk of developing cancer, and these were often initially identified by genetic linkage analysis of affectedfamilies. The size of the full repertoire of human cancer genes is a matter of speculation. However, studies in mice have suggested that more than 2,000 genes,

when appropriately altered, may have the potential to contribute to cancer development. The known cancer genes run the gamut of tissue specificities and mutation prevalences. Some, for example TP53 and KRAS, are frequently mutated in diverse types of cancer whereas others are rare and/or restricted to one cancer type. In some cancer types, for example colorectal and pancreatic cancer, abnormalities in several known cancer genes are common. In contrast, in gastric cancer, relatively few mutations in known cancer genes have been reported. Approximately 90% of the known somatically mutated cancer genes are dominantly acting, that is, mutation of just one allele is sufficient to contribute to cancer development. The mutation in such cases usually results in activation of the encoded protein. Ten per cent act in a recessive manner, requiring mutation of both alleles, and the mutations usually result in abrogation of protein function (these are sometimes known as tumour suppressor genes). Patterns of mutation differ between dominant and recessive cancer genes. Recessive cancer genes are characterized by diverse mutation types, ranging from single base substitutions to whole gene deletions, which have the common outcome of abolishing the function of the encoded protein. In each dominantly acting cancer gene, however, the repertoire of cancer-causing somatic mutations is usually more constrained, both with respect to the type of mutation and its location in the gene. Missense amino acid changes (often restricted to certain key amino acids), in-frame insertions and deletions, and gene amplification are all common mutational mechanisms for activating dominantly acting cancer genes. Most, however, are activated through genomic rearrangement. This may join the sequences of two different genes to create a fusion gene or it may position the cancer gene adjacent to regulatory elements from elsewhere in the genome, resulting in abnormal expression patterns. Most of the known rearranged cancer genes are operative in the relatively rare subset of cancers constituted by leukaemias, lymphomas and sarcomas. Recently, however, rearranged cancer fusion genes were discovered in more than half of prostate cancer cases and in lung adenocarcinomas. Their late discovery probably reflects the difficulty of identifying them amidst the jumble of passenger rearrangements present in many

cancer genomes and hints that there are many more rearranged cancers genes to be found in common cancers. Much of what we know about the biological pathways and processes that are subverted in cancer has originated from experiments exploring the functions of cancer genes. Certain gene families, notably the protein kinases, feature particularly prominently among cancer genes. Furthermore, cancer genes cluster on certain signalling pathways. For example, in the classical MAPK/ERK pathway upstream mutations are found in cell-membrane-bound receptor tyrosine kinases such as EGFR, ERBB2, FGFR1, FGFR2, FGFR3, PDGFRA and PDGFRB and also in the downstream cytoplasmic components NF1, PTPN11, HRAS, KRAS, NRAS and BRAF. Recent exhaustive mutational analyses in gliomas have indicated that almost all cases have a mutation at one of the genes on these critical signalling pathways. For some cancers, classification and treatment protocols are now defined by the presence of abnormal cancer genes. Acute myeloid leukaemia, for example, is subclassified on the basis of the presence of abnormalities involving specific cancer genes. Each subtype has a characteristic gene expression profile, cellular morphology, clinical syndrome, prognosis and opportunity for targeted therapy. Moreover, because cancer cells are dependent on the abnormal proteins encoded by mutated cancer genes, they have become targets for the development of new cancer therapeutics. Flagships for this new generation of treatments include imatinib, an inhibitor of the proteins encoded by the ABL and KIT genes, which are mutated and activated, respectively, in chronic myeloid leukaemia and gastrointestinal stromal tumours, and trastuzumab, an antibody directed against the protein encoded by ERBB2 (also known as HER2), which is commonly amplified and overexpressed in breast cancer.

The Cancer Genome Atlas (TCGA) is a large-scale, collaborative effort led by the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) to map the genomic and epigenomic changes that occur in 32 types of human cancer, including nine rare tumors. Its goal is to support new discoveries through the generation of a catalog of somatic aberrations (part

list) occurring in the different neoplasms, and accelerate the pace of research aimed at improving the diagnosis, treatment, and prevention of cancer. TCGA is a community resource project, and as such, project data is released to the public immediately after it is judged to be technically correct through quality control metrics. The information generated by TCGA is centrally managed and entered into databases as it becomes available, making the data rapidly accessible to the entire research community. By January 2015, TCGA had generated about 1.7 petabytes of data for about 11,500 cases of tumor and matching normal tissue samples. TCGA provides the cancer research community with a valuable resource. TCGA has: 1. Collected an unprecedented number of high-quality human cancer samples as well as their matching normal tissues, allowing researchers to identify important genomic changes that may play a role in the development of cancer. 2. Consistently characterized and analyzed each sample, yielding a deeper, more reliable, and broader perspective of the cancer genome compared to previous approaches of more limited scope. 3. Fostered collaborations across broad cross-sections of the cancer research community by making the data freely available in real time. The TCGA program includes a broad cross-section of the cancer research community. The TCGA Research Network includes scientists, bioinformaticians, bioethicists, physicians, nurses, advocates, and many others working collaboratively to generate and analyze the data produced in the context of the project. The TCGA Network consists of four basic components. TCGA data are available to the public and research community at large in two data repositories: the TCGA Data Portal and the Cancer Genomics Hub. These two databases and the way to access the data stored within will be discussed at length in the following sections. The goal and purpose of this chapter is to facilitate the access to the TCGA data by those who can best explore its richness and help in making the discoveries that will alleviate suffering from cancer.

2.3 Epigenetics

Epigenetics is defined as the study of heritable changes in gene expression without alteration in DNA sequences. Epigenetic gene patterns play an important role in various biological processes including embryonic development, genetic imprinting, and X-chromosome inactivation. Disruptions in these processes lead to a wide variety of pathologies including metabolic and autoimmune diseases, neurological disorders, and cancer. The key processes responsible for epigenetic regulation are DNA methylation, chromatin modification (covalent alteration in core histones), nucleosome positioning (physical changes), and posttranslational gene regulation by noncoding RNAs. Deregulation of these processes causes aberrant gene function and altered gene expression that play critical role in cancer initiation, development, and subsequent progression.

Epigenetic Regulation in Cancer are:

DNA Methylation

Histone Modification

Noncoding RNAs

Nucleosome Positioning

Chromosomal Looping

Cancer Epigenomics

Aberrant epigenomics contributes to neoplastic development by its involvement in the initiation, promotion, invasion, metastasis, and chemotherapy resistance. Reports suggest that more than

300 genes and gene products are epigenetically altered in human cancers through various epigenetic regulated mechanisms. This advancement has been possible through the advent of high throughput technology to map the human genome at single nucleotide resolution. Similar to cancer genomics, cancer epigenomics has advanced due to large-scale epigenomics design for characterization of epigenetic alterations at the global and specific gene expression level.

The first link between DNA methylation and cancer was established in 1983, demonstrating that genomes of cancer cells are hypomethylated relative to normal counterparts. Cancer cells frequently demonstrate genome-wide hypomethylation and site-specific CpG island hypermethylation in the gene promoter regions. DNA hypermethylation at specific genes typically affects promoter CpG islands and inactivates transcription. The transcriptional inactivation caused by promoter hypermethylation affects genes involved in the major cellular pathways including DNA repair, cell cycle control, Ras signaling, apoptosis, metastasis, hormone response, vitamin response, and p53 network among others. This provides tumor cells with higher growth advantage and increases genetic instability and malignant phenotype. DNA hypomethylation in tumor cells is primarily caused by loss of methylation from repetitive regions of the genome causing genomic instability and changes in gene imprinting. In cancer, hypomethylation is often associated with gain of function of oncogenes such as transcription factor c-Myc, which acts as an oncogene and is widely reported to be hypomethylated gene in cancer. Furthermore, hypomethylation at specific promoters can activate aberrant expression of oncogenes and induce loss of imprinting (LOI). The most common LOI event due to hypomethylation occurs in insulin-like growth factor 2 (IGF2), and is reported in wide range of cancers including breast, liver, lungs, and colon. Similarly, S100P in pancreatic cancer, SNCG in breast and ovarian cancers, and melanoma-associated gene (MAGE) in dipeptidyl peptidase 6 (DPP6) in melanoma are well cited examples of hypomethylated genes in cancer.

In addition to changes in DNA methylation, histone modification patterns are also altered in human tumors. Studies have demonstrated that histone modification patterns are predictive for gene expression. For example, actively transcribed genes are characterized by high levels of H3K4me3, H3K27ac, H2BK5ac, and H4K20me1 in the promoter and H3K79me1 and H4K20me1 along the gene body. Loss of acetylation is mediated by HDACs that have been found to be over-expressed or mutated in different tumor types. Aberrant expression of both HMTs and HDMs is observed in various cancer types. A recent study has described inactivating mutations in the histone methyltransferase SETD2 and in the histone demethylase UTX and JARID1C in renal carcinomas. H3 acetylation and H3K9 dimethylation can discriminate between cancerous and nonmalignant prostate tissue and H3K4 trimethylation can predict occurrence of prostate-specific antigen serum level elevation after prostatectomy for cancer. EZH2 (enhancer of zeste homolog 2) expression is an independent prognostic marker that is correlated with the aggressiveness of cutaneous melanoma, prostate, breast, and endometrial cancers. Altered epigenetic patterns in cancer cells are also associated with the deregulation of long noncoding RNAs and repositioning of chromatin-modifying complexes. For example, increased expression of the ncRNA HOTAIR was found in primary and metastatic breast tumors. Over-expression of HOTAIR, which is normally expressed antisense to the HOXC locus during development and targets the PCR2 complex to the HOXD locus, leads to different PCR2 occupancy at chromatin sites, altered H3K27 methylation patterns, and increased cancer invasiveness in breast cancer cells. Another ncRNA involved in targeting of PCR complexes to tumor-suppressor genes is antisense noncoding RNA in the INK4 locus (ANRIL). ANRIL is transcribed from the antisense strand at the INK4b-ARF-INK4a locus, which is an important regulator of cell cycle progression, apoptosis, and cellular senescence. By recruiting PCR1 and PCR2 complexes to form heterochromatin surrounding the INK4b-ARF-INK4a locus, ANRIL mediates silencing of these tumor-suppressor genes.

The new role of ncRNA has been unraveled which act as decoys for miRNA when they contain specific miRNA-binding sites. For example, the competing endogenous RNA is the transcribed PTENP1 pseudogene which contains numerous miRNA response elements also present in tumor suppressor PTEN. PTENP1 has been shown to regulate cellular levels of PTEN by detracting miRNA from PTEN mRNA and is selectively lost in most human cancers indicating its tumor suppressive function. Similarly, miRNA expression patterns also confirm the malignant state. Altered expressions of various miRNAs have been observed in some tumor types. The first association between miRNA and cancer development was described in chronic lymphocytic leukemia with chromosome 13q14 deletion. This deletion deregulates miRNA-15 and miRNA-16. Most of the targets of these two miRNAs are involved in cell growth and cell cycle. The let-7 is one of the most widely studied miRNA families in cancer. Alterations of let-7 function have been described in several human cancer types, including carcinomas of the head and neck region, lung, colon, rectum and ovary. It acts mainly as a tumor-suppressor miRNA. miRNA-145 is a well-known tumor-suppressor miRNA downregulated in many human cancers owing to aberrant DNA methylation of its promoter and/or p53 mutations. This miRNA is a pluripotency repressor which regulates silencing of OCT, SOX2, and KLF4 in human embryonic stem cells; these genes are required for cell self-renewal and pluripotency maintenance. Interestingly, it is becoming apparent that the expression of epigenetic regulatory enzymes such as DNMT, HATs, and HMTs can be controlled by miRNAs. In particular, the miRNA-29 family can directly regulate the expression of DNMTs such that downregulation of this family of miRNAs in small-cell lung cancer results in increased expression of DNMT3A and 3B causing a global genomic hypermethylation and specific methylation- induced silencing of tumor-suppressor genes such as FHIT and WWOX.

Cancer Epigenetics and Its Link to Genetics

It is evident that discrete genetic alterations in neoplastic cells alone cannot explain the process of multistage carcinogenesis. Neoplastic cells undergo number of transformations during complex phase of tumor development and progression reflected in their phenotype. This process is facilitated by altered epigenome and deregulated epigenetic mechanisms initiating genetic instability resulting in the acquisition of genetic mutation in tumor suppressor genes and activating genetic mutations in oncogenes. Moreover, epigenetic changes in tumors are generally of a clonal nature which occurs in early generation of cancer cells. It is well established that 5-methylcytosine (m5C) residues are “hot spots” for mutations, which can destabilize gene structure and function. One third of germ-line point mutations leading to human genetic diseases occur at CpGs and most of these mutations are C → T transitions. This is because m5C is highly mutable by deamination, resulting in transitional mutations (i.e., C → T) at CpGs. In view of the symmetry of these CpG motifs, the methylcytosine on the opposite strand may also be affected, leading to (G → A) changes. As a consequence, CpGs are hot spots for mutations, in a variety of genes. G → A transitions are found in 44.8 % cases of leukemia and myelodysplasia, and in 60 % of colon cancer cases. C → T and tandem CC-TT mutations are found in basal cell and squamous cell carcinomas. Methylation increases the rate of hydrolytic deamination and also increases the reactivity of neighboring guanines to electrophiles. The oxidation of m5C may contribute to the high frequency of C → T transitions at CpG sequences. Oxygen radicals can react with m5C to oxidize the 5, 6-double bonds and the intermediate product, m5C glycol, and then deaminates to form thymine glycol. Oxidative stress can contribute to tumor development not only through genetic mechanisms but also through epigenetic mechanisms. As noted earlier, the presence of hydroxyl radicals can cause a wide range of DNA lesions including base modifications, deletions, strand breakage, and chromosomal rearrangements. Such DNA lesions have been shown to interfere with the ability of DNA to function as a substrate for the DNMTs, resulting in global hypomethylation. The presence of 8-OHdG in CpG dinucleotide sequences has been shown to strongly inhibit methylation of

adjacent cytosine residues. In addition, 8-OHdG may not be recognized by proofreading enzymes and thus may persist as a mutation resulting in G → T transversions. These studies suggest that oxidative DNA damage can affect patterns of DNA methylation leading to aberrant gene expression and possibly contributing to the development of malignancy.

Conclusions and Future Directions

The importance of epigenetics in cancer has been recognized and the field has emerged rapidly over the years. Advancement in technology and the use of new high-throughput methods has made possible to study epigenetic process at the global level than a single gene. Techniques like next-generation sequencing allow studies of DNA methylation status of human cells at nucleotide resolution. In addition, high-density microarrays for miRNA profiling and ChIP-Chip and ChIP-Seq have ability to detect precisely the location of different covalent histone modifications at the global level. These epigenomics approaches have revolutionized cancer research and have expanded the understanding of tumorigenesis to provide information about epigenetic biomarkers for detection, prognosis, and therapeutic assessment. Additionally, understanding of the link between epigenetics deregulation and cancer will help in designing better treatment strategies. Moreover, the intrinsic reversibility of epigenetic modifications represents an exciting new opportunity for the development of novel strategies for cancer prevention and therapeutic intervention.

2.4 Metastasis

The spread of tumor cells from a primary neoplasm to distant organs and the development of metastasis is one of the most

devastating and fearsome aspects of cancer. Metastasis is defined as the transfer of disease from one organ, or part, to another not directly connected to it.' Despite remarkable progress in surgical procedures and adjuvant local and systemic therapy, most patients with cancer die of metastatic disease. Reasons for lack of success include the frequency of metastases at time of diagnosis, location in organs in which effective therapy is associated with undue toxicity, and the fairly large size of metastases at the time of clinical detection. Because of the potential of metastatic spread, cancer is considered to be a generalized disease. New developments in gene technology, cell, tissue, and organ culture under defined conditions, as well as progress in chemotherapy and biotherapy have focused considerable attention on the processes that differentiate benign from malignant growth, for example, invasion, dissemination, and establishment of metastases. Of interest is the establishment of several new journals in the past decade specializing in this subject: Invasion and Metastasis (1981) , Cancer Metastasis Reviews (1982) and Clinical and Experimental Metastases (1982). The phenomenon of metastasis as a clinical dilemma has engaged the interests of not only oncologists, but cell biologists, biophysicists, biochemists, and immunologists. This article will synthesize information known to date to provide a framework for the understanding of why and how tumor cells metastasize and what implications metastasis has for the clinical practice of oncology.

BIOLOGICAL HETEROGENEITY OF TUMOR CELLS

Though tumors arise from a single transformed cell, the subsequent mass that becomes clinically detectable is comprised of a heterogeneous population of malignant cells representing a wide range of cellular properties, including cell surface enzymes and receptors, morphological properties, growth properties, immunologic characteristics, sensitivity to therapeutic agents, and metastatic potential.

Diversity of cells within a tumor develops slowly. The evolution and progression to heterogeneity occurs not only in the primary

tumor but in metastases as well. Progression is characterized by a series of permanent irreversible changes that occur in each distinct subpopulation of cells. Differences in tissues, nutrients, growth factors, oxygen, hormones, enzymes and other possible tumor regulators may play a role in destabilizing tumor cells and making them susceptible to genetic changes, such as chromosomal alterations, mitotic errors, and spontaneous mutations. Such variant cells are thought to have selective growth properties and other competitive advantages over normal cells. The degree to which these diverse cells compete for necessary survival and growth factors and evade host immune responses and environmental signals determines whether they become increasingly dominant tumor cell subpopulations within the tumor.

THE METASTATIC CASCADE

It was once thought that metastasis was a chaotic, random phenomenon. Recent advances in molecular and cellular biology have shown that metastasis is not chaotic or random, but is a complex process involving a sequence of interrelated steps that, in principle, are the same for all tumors. However, numerous intrinsic properties of cancer cells and responses of the host contribute to and modulate the outcome of the metastatic process, causing different results in different individuals. The complexity of the metastatic sequence contributes to its inefficiency, as most tumor cells do not survive the process. In fact, research indicates that less than 0.01% of cancer cells that leave the primary tumor subsequently become a metastasis. Those cells that do survive the potentially lethal metastatic process have unique characteristics that distinguish them from other cells in the tumor. The metastatic process begins with the transformation of a normal cell into a malignant cell and ends with secondary tumors at distant sites. In between those events is a sequence of steps required for the formation of a metastasis. The exact sequence of these steps remains to be determined. For purposes of this article, the metastatic sequence will be oversimplified and discussed according to six major processes: 1)

local invasion and detachment, 2) dissemination, 3) arrest, 4) extravasation, 5) angiogenesis, and 6) growth and development of metastasis.

Local Invasion and Detachment

Tumor invasion begins with the direct extension of some of the tumor cells within the primary neoplasm into adjacent tissues, but eventually the cells or clumps of cells break away from the tumor mass. With the exception of cells of the immune system, normal cells do not move in and out of body tissues. Cancer cells possess the unique characteristics of motility and invasiveness that is the result of a combination of mechanical forces, decreased cell-to-cell adhesion, and biochemical factors. The force of an expanding tumor exerts pressure on adjacent tissues that separate, allowing tumor cells into interstitial spaces. However, even in experimental systems where it is known that pressure is not a factor, invasiveness of tumor cells occurs, so elements other than mechanical forces must come into play. Cancer cells do not stick to each other or to other substances as well as normal cells. This diminished adhesiveness, resulting from a myriad of cell surface alterations, contributes to a cancer cell's tendency to break away from the tumor mass.

Neoplastic transformation results in a variety of changes in the surface membrane that cause alterations in the biochemical behavior of cancer cells. Two changes notable for their impact on invasiveness of cancer cells is lost or altered fibronectin secretion and an increase in the production of proteolytic enzymes. Fibronectin is a glycoprotein found in the blood and on normal cell surfaces. It is a component of the extracellular matrix or stroma that holds cells in place in tissue, isolates tissue compartments, and influences tissue structure. It also influences organization of proteins on cell surfaces and within the cell. Hynes indicates that cancer cells stop making fibronectin, or make a defective form to which cells cannot adhere, contributing to cellular disorganization, loss of cellular adhesiveness, and increased cellular motility. Cellular proteolytic enzymes, or

proteases, have been implicated in tumor invasion and metastasis for their destructive effects on the extracellular matrix. It has been shown that cancer cells release proteases such as collagenases (specific to collagen), plasminogen activators (specific to plasmin), and lysosomal enzymes. Collagenases that degrade interstitial collagen have been identified, as well as a collagenase specific to type IV collagen, the structural element of the vascular basement membrane.

Interactions of cancer cells with extracellular matrix

There are two types of extracellular matrices: interstitial stroma supports parenchymal cells of organs and consists of types I, II, and III collagen; and basement membrane, the layer intervening between epithelium and connective tissue containing type IV collagen and laminin. Profound changes occur in the distribution and quality of the epithelial basement membrane in an undifferentiated invasive carcinoma in contrast with normal benign tissue. Invasive carcinomas consistently exhibit a defective basement membrane in tissues adjacent to the invading tumor cells in the stroma. Such defects are also present around tumor cells in lymph node and organ metastases. These defects may signal the earliest stages of invasive carcinoma. Although several benign proliferative disorders (eg, intraductal hyperplasia, fibrocystic disease) are characterized by disorganization of the epithelial stroma, a continuous membrane always separates the epithelium from the stroma. In contrast, in zones of actual microinvasion, the basement membrane is markedly fragmented or absent altogether. Defects may be due to decreased synthesis or abnormal assembly of components, or to increased breakdown by tumor or host-derived proteases. The destruction of basement membranes by invasive tumor cells represents a fundamental morphological and biological difference between malignant and benign tumors that can be exploited diagnostically.

Once tumor cells escape the primary tumor, they must interact with preexisting host basement membranes at many stages in the metastatic cascade, for example, during entry or exit from

blood vessels, invasion of muscle or nerve, or when crossing most epithelial boundaries. There are two exceptions. Tumor cells in the interstitial space do not need to cross a basement membrane when entering lymphatic circulation because lymphatic capillaries lack a formed basement membrane containing type IV collagen and laminin. Similarly, tumor cells entering the liver from the portal vein do not cross whole basement membrane to invade the liver parenchyma because normal adult liver sinusoids do not contain a formed basement membrane. Infiltration by tumor cells of extracellular tissue depends on many factors associated with properties of the tumor cell, the host cells, and the matrix itself. Liotta proposed a three-step hypothesis to describe tumor-cell invasion of the extracellular matrix. The first step is tumor-cell attachment through cell surface receptors that bind to components of the matrix, such as laminin and fibronectin. Next, the anchored tumor cell secretes proteolytic enzymes (or induces the host to do so) which can degrade the matrix. Finally, the tumor cell moves into the region of the matrix modified by proteolysis. Each of these steps will be briefly described.

In the first step, cells attach to the extracellular matrix through substances known as attachment factors. These factors form a bridge between the tumor-cell surface and other structural components of the matrix. Attachment factors may be synthesized by the attaching cell itself or the cell may use attachment factors already present in the matrix. One such factor, laminin, found in capillary basement membrane, may play an important role in a variety of biological phenomena including cell attachment, growth, morphology, and cell migration. Many types of normal and neoplastic cells contain high-affinity binding sites for laminin on their surfaces (laminin receptors). These receptors mediate adhesion of tumor cells to the basement membrane before invasion. Laminin receptors on cancer cells may be increased in number and/or have higher binding capacity than those found on normal or benign cells. For example, breast and colon carcinoma cells contain a higher number of unoccupied laminin receptors as compared with benign cells.

This abundance of free laminin receptors on the tumor cell surface may facilitate adhesion of tumor cells with host basement membranes during metastasis. Laminin represents only one of a variety of substances that mediate the attachment of invasive metastatic tumor cells to extracellular matrices. Tumor-cell invasion of the extracellular matrix requires active biochemical mechanisms. A number of research groups have proposed that invading cells produce enzymes capable of degrading the matrix. Investigators have identified tumor-derived collagenases that degrade types I, II, and III interstitial collagen, a major structural element that supports other components of the matrix, and a type IV collagenase that destroys basement membrane and is present in many highly metastatic tumor cells. Because of the complex, multistep process of invasion and metastasis, it is unlikely that one property such as collagenase production will correlate quantitatively with metastatic propensity. Although collagenase activity of malignant tumor tissue is consistently higher than in corresponding benign tissue, degradation of the extracellular matrix by enzymes may be necessary but not sufficient for tumor invasion to occur.

The final step in the invasion process is the locomotion of tumor cells. Locomotion may manifest in a “creeping motion” of isolated cells or in the moving boundary of a sheet of cells, and includes the detachment and infiltration of cells from the primary tumor into adjacent tissue and the migration of cells through the vascular wall into the circulation. Migration may be initiated by loss of intercellular adhesion or by dissociation of intercellular bonds by cell surface-associated proteases. The actual movement of cells through biological barriers is influenced by factors that function as “chemo attractants”, contributing to the directional aspects of a motile response. However, these agents are not sufficient to initiate the intrinsic locomotion of tumor cells. Studies have suggested that transformed cells produce their own (autocrine) motility factors.34 Under the influence of such autocrine motility factors (AMF), a tumor cell might move out of the surrounding host tissue and exert a recruiting effect on adjacent tumor cells. Researchers found that human melanoma

and breast carcinoma cells produce in culture such a factor that markedly stimulates their motility in a dose-dependent fashion.

Dissemination

Lymphatic

For many types of cancer, a mass in the regional lymph nodes is the first clinical sign of metastasis. Anatomic proximity explains why regional lymph nodes are such a common site of metastasis. However, it is now known that what was thought to be a simple filtering mechanism of tumor cells flowing through lymph nodes is more complex. Biochemical substances on the surface of the cancer cells, and the intactness of the host immune system may be important determinants of whether tumor cells entering a lymph node develop into a tumor mass in that node and/or subsequently enter the lymphatic system. Once tumor cells invade the lymphatic vessel, they are carried to the regional lymph node, where a number of outcomes is possible. The tumor cells can lodge in the lymph node and grow into a discernible mass or die as a result of an inflammatory response from the immune system or because the environment is not otherwise conducive to growth. Fisher and Fishers demonstrated that a significant percentage of tumor cells entrapped in a lymph node will break away and enter the lymphatic system within 10 to 60 minutes after arresting in the lymph node and ultimately enter the regional or systemic venous system. It has been shown that the lymph node is not a true mechanical barrier as once thought and that lymphatic dissemination is not separate from hematogenous dissemination, as there are numerous lymphatic-venous interconnections throughout the body. Therefore, it is likely that lymphatic and hematogenous dissemination occur concurrently.

Hematogenous

Because of the numerous interconnections between the lymphatic and vascular systems, widespread tumor cell

dissemination may result from the penetration of blood vessels or lymphatics as tumor cells pass from one system to another. Tumor cells frequently penetrate the thin walls of capillaries in the process of invasion. The cells are carried away passively or develop and grow at the penetration site with subsequent release of tumor emboli into the circulation. The rate of hematogenous spread and metastasis is correlated to the degree of tumor vascularity. Although metastasis is a major cause of death from cancer, it has been shown that only 1% or less of the millions of tumor cells that escape from the primary tumor into the circulation survive to become a metastasis. This high cellular death rate may be the result of mechanical shear forces, inadequate attachment, insufficient oxygenation, or destruction of potentially metastatic cells by circulating host immune cells. Thus, the presence of tumor cells in the circulation does not necessarily mean that a metastasis will result. The survival of tumor cells may be enhanced by aggregation with one another or with host cells such as platelets or lymphocytes. Some tumor cells arrest in a blood vessel where they either stay or invade through the vessel wall to the target organ tissue.

Arrest

Having survived entry into and passage through the circulatory system, to become a metastatic tumor requires that cells arrest in the vascular epithelium before they can invade the vascular wall and interstitial stroma to enter the target organ. Much research has been conducted to determine factors that contribute to arrest of tumor cells at specific sites. As described earlier, the most frequent site of blood-borne metastases is the first capillary bed encountered by circulating cells. Thus, anatomic location in relation to blood flow through specific vascular pathways is an important factor in the site of metastasis for certain types of cancer such as lung, colorectal, testicular, prostate, breast, head and neck, ovarian, and sarcoma. However, metastasis often occurs in distant organs and many tumors tend to display relatively specific patterns of organ distribution unrelated to patterns of blood flow.

Extravasation

Once tumor cells arrest in the vascular endothelium, they must

actively invade their way back through the vascular wall into the interstitial stroma of the target organ. Chew et al have shown that while disseminating through the bloodstream, tumor cells associate themselves with a variety of host cells that aid in their survival and the process of reinvasion. For example, platelets aid tumor cells in attaching to the vascular epithelium by making them “stickier,” and fibrin is part of the tumor cell-platelet complex that causes damage to the basement membrane of the vascular epithelium allowing tumor cells to escape through to target organ tissues. In addition, fibrin may form a protective shield around the tumor cell, protecting it from host immune cells and the turbulence of blood flow.

Angiogenesis

Once tumor cells reach the interstitial stroma of the target organ, the subsequent growth and development of a metastasis is first dependent on the development of a vascular supply to allow nutrients and waste products to diffuse in and out of the mass. In 1971, Folkman et al reported the isolation of a substance called tumor angiogenesis factor (TAF). TAF, produced by a variety of solid tumors, was found to diffuse from intact tumor cells and induce capillary endothelial cell growth through DNA synthesis in cells as far as 3.5 mm from the site of release. In recent years, many angiogenic factors have been purified including acidic and basic fibroblast growth factors, angiogenin, transforming growth factors alpha and beta, prostaglandins, and certain serum degradative proteases. Thus, TAF released by tumor cells causes the host to produce blood vessels for the tumor aiding its growth and development. The greater the tumor’s vascularization, the more likely it is to metastasize. A variety of normal processes in the adult involve angiogenesis, including placental nourishment of the fetus, maturation of the corpus luteum, wound repair, and delayed hypersensitivity reactions. Angiogenesis is also observed in pathological conditions such as tumors, keloids, and chronic inflammatory processes. The process of capillary formation in cancer, known as neovascularization, is markedly different from the vascular regeneration that occurs in small wounds. Traumatic injury normally evokes an inflammatory response in

which exudates of fluid, leukocytes, and fibrin appear in the wound, with dilation of adjacent vessels. Gradual recovery begins with the development of collateral circulation around the wound, and eventually, new capillaries proliferate into the wound. However, as healing progresses, these capillaries begin to differentiate into arterioles or venules, or to regress. In contrast, tumor neovascularization begins immediately, increases continuously, and newly generated vessels at the periphery of the tumor do not differentiate or regress.

Growth and Development of Metastases

The ability of a metastasis to grow and develop depends largely on the environment in which it is located. In 1873, Paget introduced the “seed and soil” hypothesis that site-specific metastasis was related to the existence of an environment (the “soil”) in which compatible tumor cells (the “seed”) could proliferate. More recently, researchers have corroborated that both the “seed” and “soil” are important determinants of where metastatic deposits flourish. Host factors, not well understood, may also contribute significantly to patterns of metastasis. Immune status, hormones, age, genetics, blood flow, and vascularity have all been determined to play a role in metastasis.

2.5 Metabolism

Cancer is a major disease burden that affects both genders and causes considerable worldwide mortality and morbidity. Detection at an early stage and understanding the metabolism of cancer, both during progression and regression of the disease, is essential for better treatment outcomes. Also, an in-depth understanding of the pathophysiology of cancer is essential in order to develop patient-tailored therapy. The study of cancer metabolism, using various techniques and methods, is an important area of research. Regarding this, several studies have

addressed the potential role of various MR methods in improving our understanding of the different aspects of tumor metabolism related to the progression and aggressiveness of malignancy, and the changes that occur during therapy. In recent years, the area of metabolomics has also found widespread use in the diagnosis of various disease processes, such as transcriptomics and proteomics. It measures small molecules (low molecular weight metabolites) within cells, tissues or whole organisms. Both qualitative and quantitative changes of metabolites in real time can be monitored using these approaches. These may arise due to alterations in metabolic pathways that occur because of genetic modification, physiological and/or pathophysiological stimuli. Of the various techniques used to study metabolomics, mass spectrometry and NMR have been widely employed to detect and quantify metabolites. Notably, MR methodologies are being continuously developed at a very rapid pace to understand cancer metabolism and to identify new biomarkers for early disease diagnosis and therapy monitoring. The journey to produce this special issue began in April 2017 at the editorial board meeting of NMR in Biomedicine at the International Society for Magnetic Resonance in Medicine (ISMRM) meeting in Honolulu, Hawaii. It was felt that an issue that highlights the role of different MR methodologies, both in human and animal models, in understanding the metabolism of cancer and the potential of metabolomics in cancers, would be timely. One of the hallmarks of cancer is the activated choline metabolism, characterized by increased levels of phosphocholine, glycerophosphocholine and total choline-containing compounds. The alterations in the choline metabolism of cancer have been widely studied using magnetic resonance spectroscopy (MRS), and the majority of those studies have focused on phosphocholine and the Kennedy pathway. The importance of glycerophosphocholine in cancer metabolism has been realized, and the first review by Sonkar et al presents a summary of recent works examining the role of glycerophosphocholine in cancer and its detection by MRS. Another hallmark of cancer is lipid metabolism, which is altered during malignant transformation and during therapy. The assessment of lipids would thus be valuable. MRS and other molecular imaging methods have been

used to study oncogenic transformations and examine the changes in lipids in response to a wide array of anticancer strategies, including chemotherapy, radiation therapy, signal transduction inhibitors, gene therapy and immunotherapy, or a combination of these strategies. Arlauckas et al review the role of lipid metabolism in cancer and its response to therapy, and also summarize the current field and areas of unmet needs. The measurement of neuronal and glial energy metabolism, and glutamate and GABA neurotransmitter cycling in the brain, by the use of 13C MRS and 1 H- 13C MRS in human and animal models, are reviewed by Rothman et al. The neuroenergetic and neurotransmitter cycling pathways studied by 13C MRS and the experimental validation of these measurements are presented. The review also summarizes the results of applications of 13C MRS in neurological and psychiatric diseases, diabetes, Alzheimer's disease, healthy aging and brain cancer. Limitations of the methods used and a summary of recent progress are also provided. Breast tumors being heterogeneous, their metabolism differs from other tumor subtypes, and also during disease progression. Studies have documented that the patient's outcome from therapy depends on several factors such as hormone receptor status, lymph node involvement and tumor grade. Furthermore, the response variation among patients following therapy may be related to how the cancer progresses and alters its metabolism. It is likely that such metabolic alterations are related to drug resistance observed in patients. Thus, the study of breast cancer metabolism is essential for early detection, diagnosis and response evaluation of tumors to interventions and therapies. Jagannathan, in his review, describes the role of various in vivo MR methodologies used in the study of breast cancer metabolism. Prostate cancer in men is extremely variable in its aggressiveness and progression. Low- or intermediate-risk patients with localized cancer face a dilemma in selecting appropriate therapies. For example, whether to undergo aggressive treatments like surgery or whole-gland radiation therapy with their associated complications, or undertake active surveillance without treatment. In this direction, focal therapies like high- intensity focused ultrasound

(HIFU) have recently been the treatment of choice, thereby reducing the risk of complications of radical therapy.
Lee et al review the role of the hyperpolarized (HP) dual agent (13C pyruvate and 13C urea) 13CMRI/mp- 1 H MRI approach, used to study the temporal changes in prostate cancer metabolism and perfusion. These were evaluated after exposure to ablative and sublethal doses of HIFU in a transgenic adenocarcinoma of a mouse prostate model and a FUS applicator designed for murine studies. Glycolysis is fundamental to various metabolic processes, and abnormality in glucose metabolism may be related to a variety of pathological conditions. For example, a characteristic feature in rapidly growing cells is the observation of higher aerobic glycolysis. In fact, using hyperpolarized 13C-NMR, it is possible to monitor glycolysis and the pentose phosphate pathway in real time. In their review, Singh et al discuss the application of various HP 13C-labeled metabolites in real-time monitoring of glycolysis and related metabolic processes in normal and diseased tissues. MRS is the method of choice in many studies to evaluate cancer metabolism, in addition to MRI methods such as DWI, dynamic MRI and chemical exchange saturation transfer (CEST) MRI. Julià-Sapé et al investigate the potential, from 2016 onwards, of MRSI for studying in vivo cancer metabolism-derived metrics. Their review covers the application and the limitations of single-voxel and multi-voxel MRS (MRSI) in various cancers of the brain, breast and prostate. They point out that future applications of MRSI will include the use of novel dynamic metabolic methods like hyperpolarized substrates. This is followed by a review by Morze and Merritt, who describe targeting cancer metabolism with HP 13C MRI. In their extensive review the authors discuss both the technical challenges and the biochemical aspects of HP experimental design to study the aberrant glycolysis and central carbon metabolism of cancer. Next, Kishimoto et al summarize the applications of MR-based metabolic imaging methods, focusing on pO2 and pyruvate-to-lactate conversion. For this purpose, electron paramagnetic resonance and proton-electron

double-resonance imaging have been developed to study metabolic profiling, and have been successfully used to monitor pO2 in cancerous tissue in animal models. The use of hyperpolarized 13C MRI to visualize the activity of the glycolytic pathway is reviewed, a method which is almost ready to be brought into the clinic. Goldenberg and Pagel review the application of CEST-MRI in the study of cancer metabolism. There are many CEST-MRI acquisition methods that are used to characterize tumor metabolism, including GluCEST, CrCEST and CatalyCEST. The uses of these techniques are described along with the associated technical challenges involved in each method. Following these reviews on preclinical and clinical systems, Penet et al review cell line studies using MR. It is known that cancer shows spatial and temporal heterogeneities. Further, it is important to study how cancers function under abnormal physiological environments like hypoxia and acidic extracellular pH. Such studies help us to understand cancer invasion and metastasis, and to identify effective treatment strategies. In their review, Penet et al discuss the application of MRI and MRS of intact perfused cancer cell metabolism, their invasion, and their interactions with stromal cells and the extracellular matrix. Complementary to these reviews, this special issue contains two original research contributions. The first is by Kemp et al, wherein the authors report the shortening of apparent relaxation time of inorganic phosphate as a breast cancer biomarker. The apparent transverse relaxation time of inorganic phosphate in breast cancer tissues is shorter than that in healthy fibroglandular tissues by a factor of greater than two. This is attributed to the effect caused by the upregulation of glycolysis in breast cancer tissue leading to the interaction of inorganic phosphate with the GAPDH enzyme that forms part of the reversible pathway of exchange of inorganic phosphate with γ-ATP. The second original research contribution is by Canes et al regarding the potential of using a multimodality MR approach for noninvasive early assessment of trabectedin (Trab) efficacy in epithelial ovarian cancer. They combined functional MRI and

MRS (in vivo and ex vivo including metabolomics) to identify new pharmacodynamic markers of Trab effectiveness. Their study identified Trab-induced alterations in lactate and phosphocholine content, and found that the presence of necrosis and increased apparent diffusion coefficient are associated with the tumor growth reduction in epithelial ovarian cancer models. The last three reviews of this special issue concern the application of NMR metabolomics in the study of cancer metabolism. The first is by Ranjan and Sinha, who examine the potential of NMR metabolomics in identifying key metabolic players in the etiology of cancer. Details of various NMR techniques as applied to solids, semi-solids and solution phases, and the statistical analysis used in the metabolomics of various diseases, are presented with a focus on the NMR methodology advances and recent developments. The next review is by Giskeødegård et al, wherein the current status of NMR metabolomics of biofluids is discussed. The focus is on the details of NMR pertaining to cancer detection, risk assessment, disease characterization and prognosis, and treatment monitoring. The association between biofluid metabolomics and cancer progression suggests that NMR metabolomics is a useful method for gaining information of prognostic or predictive value. Finally, Dinges et al review the field of high-resolution magic angle spinning (HRMAS) MRS-based metabolomic investigations of human cancers using univariate/multivariate analysis.

2. Metabolic Characteristics of Cancers

Genetic mutations confer the capability to bypass cell–cell contact inhibition and for the growth factor-orchestrated proliferation of cancer cells. However, poor vascularization in the tumor microenvironment induces chronic nutrient deprivation and reduced oxygen concentrations . To survive and adapt to these harsh environmental stresses, cancer cells modify their metabolic pathways to capture external metabolites and maximize the efficiency of metabolic enzyme activities .

2.1. Glucose Metabolism

After the Warburg effect was revealed, studies have demonstrated that glucose metabolism is the key source to provide metabolic carbon in cancer cells . When glucose enters the cytoplasm, it can be used as fuel by glycolysis, the hexosamine synthesis pathway (HSP), the pentose phosphate pathway (PPP), or the serine biosynthesis pathway. Each metabolic process provides precursors or intermediates (e.g., NADPH, nucleotides, pyruvate, amino acids, and methyl groups) for other metabolic pathways and cellular reactions. Therefore, the maintenance of stable glucose metabolism is an important requirement of cancer cell survival and cancer progression Glycolysis supplies various carbon intermediates and generates ATP and NADH. Oncogenic mutations have been shown to activate glycolytic enzymes. Glucose enters the cell via glucose transporter (GLUT) proteins. In the cytoplasm, glucose is phosphorylated by hexokinases (HKs) and remains trapped inside the cell. Through glycolysis, glucose is metabolized to the final product, pyruvate. During this process, the oncogenes c-MYC, KRAS, and YAP upregulate GLUT1 expression in cancer cells .The overexpression of YAP and loss-of-function mutations in p53 increase GLUT3 expression, which causes its accumulation in the plasma membrane .The phosphoinositide 3-kinase (PI3K)/AKT pathway is hyperactivated in cancer cells, and it upregulates HK2 activity by increasing mitochondrial HK association . Cancer cells rely on aerobic glycolysis to fulfill metabolic requirements. As a result, lactate dehydrogenase (LDH) catalyzes pyruvate to lactate instead of acetyl-CoA, which can otherwise be used as TCA cycle intermediate. LDH has two isoforms, LDHA and LDHB. Both enzymes can catalyze both the pyruvate to lactate reaction and the reverse reaction. However, in various cancer cell lines, the LDHA isoform is highly expressed because LDHA prefers the pyruvate to lactate transition . HIF-1a induces LDHA activation in hypoxic tumor microenvironments . During this step, NADH, which is a byproduct of glycolysis, is oxidized to NAD^+. NAD^+ regeneration maintains glycolysis.

Glucose-6-phosphate (G6P) is produced by HK. G6P can initiate the PPP, which has two distinct phases, oxidative and non-oxidative. The oxidative phase produces NADPH and ribulose-5-phosphate (R5P); the non-oxidative phase produces only R5P, the precursor for nucleotides [20]. NADPH is necessary for fatty acid synthesis. NADPH also has reducing power, which is required to regenerate glutathione (GSH) from glutathione disulfide (GSSG). Given that cancer cells are exposed to chronic metabolic stress, control of cellular reactive oxygen species (ROS) levels is important to avoid negative outcomes including apoptotic cell death [21]. Hence, the PPP is critical for maintaining redox homeostasis and protecting cancer cells from oxidative stress. Several oncogenic proteins upregulate PPP influx. In cancer cells, hyperactive PI3K/AKT and mTORC1 signaling increase the expression of rate-limiting enzymes in the PPP (e.g., G6PD and RPIA) via sterol regulatory element-binding protein 1 (SREBP1) activation . Aberrant AKT activation in cancer cells activates the transketolase enzyme in the PPP via direct phosphorylation . Oncogenic c-Myc upregulates PPP influx . Finally, PPP activity increases when the loss-of-function mutation of p53 cannot bind and inhibit G6PD .

The HSP provides UDP-GlcNAc, which has an important role in protein post-translational modifications, such as glycosylation. Because GlcNAcylation regulates protein stability, GlcNAcylation of p53 protects the protein from phosphorylation-mediated protein degradation . mTORC1/MYC hyperactivation increases HSP activation in breast cancer cells by upregulating O-GlcNAc transferase (OGT) .

The serine biosynthesis pathway diverges from the glycolysis pathway at the level of 3-phosphoglycerate (3-PG). Phosphoglycerate dehydrogenase (PHGDH) is the first enzyme of the serine biosynthesis pathway and is the rate-limiting enzyme that converts 3-PG to 3-phosphohydroxypyruvate. During this metabolic process, phosphoserine aminotransferase (PSAT1) generates α-ketoglutarate (α-KG) as a reaction byproduct. Thus,

the serine biosynthesis pathway can provide a TCA cycle intermediate for further ATP production and anabolic metabolism . The final products of the serine synthesis pathway (i.e., serine and glycine) are precursors for other metabolic pathways, including folate metabolism and methionine metabolism. NADPH and methyl groups are generated via folate and methionine metabolism. Consequently, in addition to amino acids, the serine biosynthesis process produces reducing potential and methyl groups that are involved in post-translational modification and epigenetic regulation . PHGDH is genetically and transcriptionally upregulated in diverse cancer cell lines. The PHGDH gene is amplified and enzyme expression is elevated, especially in breast cancer cells . c-Myc transcriptionally upregulates PHGDH and other serine biosynthesis enzymes, including PSAT1, phosphoserine phosphatase (PSPH), and serine hydroxymethyltransferase 1 (SHMT1) . Therefore, glycolytic intermediates can branch off into key biosynthetic pathways to generate nucleotides, amino acids, and fatty acids that are essential to meet the increased biosynthetic needs of cancer cells.

2.2. Glutamine Metabolism

Glutamine is the most abundant amino acid in plasma. It supplies carbon and nitrogen by participating in various cellular reactions. Glutamine is critical for cancer cell proliferation because nitrogen is an essential metabolite for nucleotide biosynthesis and glutamine is a precursor for synthesis of other non-essential amino acids (NEAAs) and fatty acids .

Glutamine is imported into the cytoplasm via the glutamine transporter, ASCT2 (i.e., SLC1A5). Because glutamine is the major nitrogen donor, various oncogenic signals promote ASCT2 activity. For example, oncogenic c-Myc and n-Myc upregulate ASCT2 expression via ATF4 in neuroblastoma cells and promote glutamine uptake . mTORC1 induces ASCT2 expression . Tumor microenvironment factors promote glutamine transporter expression. The inflammatory cytokine, IL-4, binds its receptor

and induces c-Myc transcription in breast cancer cells . Lactate is the final product of aerobic glycolysis. It is imported by MCT-1 transporter and stabilizes c-Myc to increase ASCT2 expression . Ras-transformed cancer cells use extracellular vesicles to capture external plasma proteins via micropinocytosis .

Imported glutamine has two possible fates. First, glutamine can serve as the nitrogen donor for amino acid and nucleotide biosynthesis. Glutaminase (GLS1/2) enzymes catalyze glutamine to glutamate and produce ammonia (NH_3). Glutamate can also be converted to the TCA cycle intermediate, α-KG, and produce NH_4^+. This ammonia group can be transferred via carbamoyl phosphate synthetase and phosphoribosyl pyrophosphate synthase, which are rate-limiting enzymes of the pyrimidine and purine synthesis pathways, respectively . Nitrogen from glutamine is a substrate for transaminases to synthesize NEAAs (e.g., alanine, asparagine, and serine). The microRNAs miR-23a and miR-23b inhibit GLS activity by targeting the 3′-UTR region of GLS mRNA. However, in cancer cells, c-Myc activation inhibits miR-23a/b and promotes GLS translation . Moreover, PI3K/AKT axis and downstream mTOR pathway hyperactivation induce glutamate dehydrogenase (GLUD) expression by inhibiting SIRT4. This inhibition PARylates (ADP-ribosylation) and inhibits GLUD activity . Conversely, in pancreatic cancer cells, KRAS mutation downregulates GLUD activity and upregulates the aspartate synthase (GOT1) enzyme to provide NADPH for the proper maintenance of redox homeostasis .

The second fate of imported glutamine occurs when it serves as a carbon donor for fatty acid synthesis. Because the plasma membrane and subcellular organelles consist of lipid bilayers, de novo lipid biosynthesis is required for cell proliferation. Hence, lipid biosynthesis is upregulated in various types of cancers (e.g., prostate, lung, and stomach) . Under normoxic conditions in normal cells, most acetyl-CoA, which is a precursor for fatty acid synthesis, comes from glycolysis. However, because of aerobic glycolysis, cancer cells usually transform pyruvate to lactate

rather than acetyl-CoA. Under these circumstances, most of the acetyl-CoA in cancer cells is acquired from the glutamine-TCA cycle axis . Glutamine is catalyzed to α-KG, which then enters the anabolic phase of the TCA cycle to produce citrate. Citrate translocates from the mitochondria to the cytoplasm and is catalyzed to acetyl-CoA by the ATP citrate lyase (ACLY) enzyme. Thereafter, fatty acid synthase (FASN) mediates long-chain fatty acid synthesis.

Glutamine fuels TCA cycle intermediates, which are emerging as mediators of malignant transformation in cancer. As a result of aerobic glycolysis and OXPHOS, glutamine becomes the principal source of NADH and $FADH_2$ in cancer cells . However, OXPHOS inevitably produces ROS that might induce DNA damage and oxidative stress . Hence, oncogenic mutation induces diverse NADPH-providing mechanisms that confer sufficient reducing power to manage ROS levels .

2.3. Fatty Acid Metabolism

Recent studies underscore the importance of fatty acid metabolism in cancer progression. Fatty acids not only have roles as structural components but are also secondary messengers (DAG and IP_3). Thus, fatty acid synthesis is vital for cellular response and proliferation. Fatty acid synthesis requires significant amounts of NADPH, which is crucial for redox homeostasis. Therefore, it is regulated by various signal pathways to maintain the balance between redox homeostasis and cell growth .

Fatty acid synthesis is coordinated by SREBPs, which are transcription factors for lipid biosynthesis enzymes. SREBPs are synthesized and sequestered in the endoplasmic reticulum as inactive precursors .When cellular lipid levels are low, Golgi-associated MBTPS1/2 protease cleaves SREBPs at the N-terminus. The cleaved product is translocated into the nucleus, where it binds to the SRE protein and induces target gene expression . In the nucleus, GSK3β inhibits SREBP stability via FBXW7-mediated ubiquitination . However, aberrantly activated

PI3K/AKT and mTORC2 signaling inhibit GSK3β to allow for the higher expression of SREBP in various cancer cell types .As a direct target gene of SREBP, ACLY enzyme converts citrate to acetyl-CoA at the very first step of fatty acid synthesis. E3 ligase UBR4 and Cullin3-KLHL25 ubiquitinate and destabilize ACLY .ACLY acetylation via p300 inhibits ubiquitination and increases its stability ; SIRT2 deacetylates and destabilizes ACLY . After ACLY produces acetyl-CoA, acetyl-CoA carboxylase (ACC) converts acetyl-CoA to malonyl-CoA. ACC is a well-known target for AMP-activated protein kinase (AMPK), a master regulator of energy homeostasis. However, some lung adenocarcinoma cell lines have mutant liver kinase B1 (LKB1), which is an upstream regulator of AMPK. These cell lines have a constitutively active state of ACC and increased fatty acid synthesis.

Fatty acid synthesis is an oxygen-consuming process. Therefore, cancer cells try to compensate for fatty acid synthesis by upregulating external lipid uptake instead of using de novo fatty acid synthesis. This upregulation especially occurs during metabolically challenging situations (e.g., hypoxia or nutrient deprivation). In breast cancer cells, HIF-1α promotes fatty acid uptake by increasing expression of the fatty acid-binding receptor proteins FABP3, FABP7, and ADRP . KRAS activation facilitates macropinocytosis, which promotes extracellular lipid uptake and lysosomal degradation. Cancer cells use these mechanisms to overcome metabolic hurdles that restrict metabolite synthesis.

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Chapter 3: Causes

The study of cancer in human populations has contributed most obviously to knowledge of cancer causes. A few substances were first identified as carcinogens in experimental animals and subsequently have been shown to be active in humans--vinyl chloride and chloromethyl methyl ether are examples. They are responsible for no more than a tiny fraction of human cancer. Many other substances identified as cancer initiators or promoters in other species have not yet been shown to increase cancer risk in humans, despite substantial exposure of some groups of people--2,3,7,8-TCDD ('dioxin') is a notable instance. Cross-species concordance for carcinogenicity, while it no doubt exists to some extent, has not been a major contributor to knowledge of cancer causes in humans. On the other hand, the study of cancer in human populations has made tremendous contributions--to knowledge not only of causes but to knowledge of causes that can be controlled. Pre-eminent, of course, is the recognition of the role of the cigarette in the pandemic of lung cancer which now covers most of the world. Although some developed countries have made progress towards the control of this disease (Sweden and Great Britain are exemplars in this regard) it is a pandemic which will be with most of the world for at least the next 50 years. For each year that the population studies of the 1950s could have been advanced, many millions of premature and ugly deaths could have been avoided. Perhaps we should explain briefly that we use the word 'population' in a broad sense. 'Population studies' in our usage are studies of disease in groups of individuals, whether the groups be political entities (e.g. nations), formal associations (e.g. trade unions), or simply groups assembled ad hoc (e.g. the patients seen by a

particular physician). Such studies take advantage of what Willcox called "the uniformity and predictability of many important biologic phenomena taken in the mass, some of us hold that this predictability exists for all biologic phenomena--not only the important ones but it is not worth the time to argue about that; let us proceed by taking advantage of the instances where it has been demonstrated. In the context of causes of cancer, population studies have detected, and at least partially quantitated, the roles played in human cancer of tobacco, asbestos, infection with HepatitisB virus, sunlight, ionizing radiation, benzene and a few other chemicals. If exposure to these agents were eliminated (or in the case of sunlight and ionizing radiation, attenuated) human cancer rates would probably be 30 to 40% lower than they are today. There is still a long way to go. Although much is known of risk factors associated with major cancers, such as those of the breast and of the gastrointestinal tract, no practical means of prevention yet has been identified. For cancer of the liver, probably the most frequent fatal cancer world-wide, although at least one major cause has been identified the efficacy of vaccination against hepatitis-B virus as a preventive remains to be demonstrated. But there are values to the study of cancer in populations beyond those having to do with knowledge of cancer causes. The lion's share of the resources that society devotes to the fight against cancer are directed towards the treatment of persons who already have the disease. Dramatic cures are obtained, and in recent decades carefully conducted randomized trials have demonstrated that persons who get medical care do live a little longer, on average, as well as feel substantially better. But we will not know the average costs and benefits of clinical efforts to individual patients until we have studied these costs and benefits in populations. Cancer Causes and Control does not seek reports of clinically-oriented therapeutic trials, randomized or not, but is interested in reports of trials or studies which evaluate measures which have the potential to influence disease rates in the population. Finally, population studies help determine not only

how resources should be allocated to achieve a particular objective (in this case the prevention or cure of cancer) but also what proportion of societal resources should be directed to that specific purpose rather than to any or all of the many other responsibilities that modern society assumes. One has reason to be skeptical about the extent to which facts influence policy decisions and must recognize that policy decisions in regard to health are not immune from the value judgments, prejudices, and even personal ambitions, that we expect in policy decisions in other areas. Nevertheless, to the extent that facts influence policy professionals have a responsibility to see that the facts used are accurate and timely. We anticipate that the reports published in Cancer Causes and Control will help in this regard.

3.1 Chemicals

The causes of cancer are many and varied. In the minds of the lay public, cancer is caused by environmental pollutants in our food, water, and air. This simplistic view of cancer causation may contribute to the misconception that we have no control over our own cancer risk. Although there certainly are carcinogens in the air we breathe, the water we drink, and the food we eat, these are insignificant compared with those things that we can control in our environment.

Carcinogens are substances that, when introduced into a cell, cause changes in the structure and function of the cell that lead to cancer. Carcinogens are classified as chemical, radiation, or viral, and their effects may be enhanced or reduced by host factors such as genetic susceptibility. There are two ways that substances are identified as carcinogens. First, epidemiological studies may show patterns of tumor development in certain populations that suggest causality. Second, animal studies may show a cause and effect relationship between a suspected substance and a tumor. Unfortunately, it is almost impossible to estimate the degree of human risk using evidence from animals because of species-specific responses. However, a combination of

the several sources of data may provide important information about which substances are carcinogenic. Not only are carcinogens often species-specific, they are also often disease-specific. Substances that greatly enhance the risk of one type of cancer may have little or no effect on other types. Thus, cancers of different organs should generally be considered as independent diseases with different causes.

It is now believed that carcinogens work by interaction with existing proto-oncogenes and cancer suppressor genes in normal cells. All animals contain in their cells the genes to produce tumors when these genes are mutated. Proto-oncogenes are mutated and "switched on" by carcinogens such as chemicals, radiation, and viruses.

STAGES OF CARCINOGENESIS

It is useful to describe carcinogenesis as taking place in three stages: initiation, promotion, and progression. Initiation occurs when a chemical, physical, or biological agent damages DNA. This damage may be reversible or may lead to a genetic mutation if not repaired. Such a mutation alone may not lead immediately to malignant growth; however, the cell may become susceptible to promotion at a later time. Cells that are initiated may or may not become malignancies. Promotion is usually the result of a second factor acting on the initiated cell and was described by Rous and Kidd in 1941 in rabbits. Tar applied to the ears of the rabbits was the initiator and wounding of the tar-treated areas was the promotor. The combination led to the development of neoplasms along the edges of the wounds. Later, Mottram reported initiating tumors in mice with benzopyrene and then promoting the cellular changes with croton oil, a known irritant. Mottram reported a much higher rate of skin tumors when both initiator and promoter were used than when the initiator was used alone. Thus, agents that are carcinogens are known as initiators, and other agents capable of inducing neoplastic transformation only when applied after an initiator are known as promotors. Some carcinogenic agents are known as complete

carcinogens because they can both initiate and promote neoplastic transformation. Initiation is dose-dependent; some complete carcinogens will only initiate in low doses but not promote. Irreversibly initiated cells usually do not display their changes and so are not detectable until they are exposed to a promoting agent. The changes in cells that have undergone promotion are usually grossly visible. Cells that are irreversibly initiated may be promoted even after long latency periods. The latency period or tumor induction time may be an extended period in humans. The latency period varies with the type of agent, the dosage, and the characteristics of the target cells. There is a clear relationship between time sequence and the effects of tumor initiation and promotion. It is believed that promoting agents work by changing the expression of genetic information within the cell, increasing DNA synthesis, increasing gene amplification (increasing the number of copies of a particular gene), and altering intercellular communication. Some hormones have been shown to be very effective promoting agents in humans. For example, estrogen has been linked to liver adenomas, breast cancer, and endometrial cancer. Evidence of the effects of estrogen has been gained not only in animal trials but also from experience with women who have had reduced incidence of breast cancer following oophorectomy and anti-estrogen therapy. Asbestos is believed to be a promoting agent in tracheal and bronchogenic carcinomas among smokers. However, asbestos is believed to be an initiator of mesotheliomas even with brief exposure. Exposure to other promoting agents is, for the most part, under the control of the individual. For example, dietary fat, cigarette smoke, and alcohol are believed to act as promoting agents. The third stage of carcinogenesis is progression. Progression involves morphological and phenotypic changes in the cell and is associated with increased malignant behavior leading to invasion of surrounding tissue and metastasis to distant body part.

CHEMICAL CARCINOGENESIS

Historical Perspective: In 1775, Sir Percival Pott, an English

physician, noted that men who had been chimney sweeps as young boys were much more likely to develop scrotal cancer. Pott did not recommend any preventive action, but in 1778 the Danish Chimney Sweepers’ Guild recommended daily bathing. A century later there was a measurably higher rate of scrotal cancer in England where no change had been recommended compared with other parts of Europe. In 1915, the first evidence of carcinogenesis gained through laboratory experimentation was reported. Yamagiwa and Ichikawa, Japanese pathologists, applied coal tar to the ears of laboratory animals, inducing skin tumors. Thus, began the modem approach of combining laboratory research with epidemiological evidence to understand chemical carcinogenesis.

Mechanisms of Action: Results of the work of Miller and Miller led to the theory that many carcinogens are actually precarcinogens that must be somehow metabolized by the cell in order to exert their effect. It has been proposed that chemical carcinogens may be converted into chemicals with electron-deficient sites (electrophilic reactants) that exert their biological effect by interaction with DNA. In addition, during the metabolism of the carcinogen, free radicals may be formed that carry no charge but that do possess a single uppaired electron, making the free radical extremely reactive. Antioxidants such as vitamins A, C, and E inhibit the formation of free radicals and thus also may inhibit the damaging effect of many chemical carcinogens on DNA. Because the majority of chemical carcinogens must be metabolized in the cell before exerting their carcinogenic activity, substances that are carcinogenic for one species may not be carcinogenic for another because of the differences in metabolism among species. Although both saccharin and dioxin in insecticides are promoters of cancer in experimental studies in animals, they have not been shown to have the same effect in humans. Although it is not known to be a carcinogen in lower animals, benzene is known to induce leukemia in humans.

Categories of Chemical Carcinogens: Many agents have been called chemical carcinogens. Some have been confirmed to be carcinogenic through research, but many have not. Dietary carcinogens. Although many Americans believe that they are helpless to prevent cancer, experts now believe that many cancers could be avoided by changes in diet. Strong evidence may be found in the link between certain elements in the diet and cancer incidence. One area of concern among the lay public is fear of ingesting powerful direct-acting carcinogens. Although there are carcinogens in natural foods and plant products, these are not a major risk factor for cancers in the United States. Of some concern are the precursors of carcinogens that may be ingested. For example, benzopyrene may be produced when meat or fish is charcoal broiled or smoked or when any food is fried in fat that has been used repeatedly. Therefore, benzopyrene predisposes to stomach cancer, a type of cancer that is in the decline in the United States. Some ingested products provide the substrates for the formation of carcinogens in the body. For example, the powerful carcinogens known as nitrosamines may be formed from nitrites derived from nitrates that are ingested in vegetables and in foods to which nitrates have been added as a preservative. Foods preserved in this way include bacon and ham. Formation of the nitrites is assisted by tobacco smoke and requires either bacterial assistance or a mildly acidic environment. Formation of these nitrosamines is inhibited by the presence of antioxidants such as vitamin C in the stomach.

A significant area of concern at present is the production of carcinogens by microorganisms in stored foods. For example, aflatoxin, a product of the Aspergillisflavus fungus, is present in moldy peanuts, corn, and other foods that are stored in hot, humid climates. Aflatoxin is one of the most powerful liver carcinogens, and the presence of the hepatitis B virus appears to greatly enhance this risk. Seen primarily in tropical countries, cancer of the liver is relatively rare in the United States accounting for less than 1% of cancer deaths. The dietary

element thought to have the greatest impact on cancer incidence in the United States is fat. It is believed that certain fats may contribute to the production of carcinogens by increasing the amount of bile acids and cholesterol metabolites in the feces. Substances like deoxycholic and lithochohc acid are found in the stools of Western populations who are known to have a higher risk of colon cancer. These substances are found in lesser amounts in the stools of Asians and Africans in whom colon cancer is known to be much less prevalent. Larger amounts are also found in the stools of patients who have polyps in the colon. These substances have been experimentally increased in humans by giving them high-fat, high-meat diets. Low levels of serum cholesterol have been associated with colon cancer.

Fiber in the diet is believed by some to exert its effect by affecting the transport and concentration of colonic carcinogens. Bulk in the diet makes feces move more quickly through the alimentary canal, thus decreasing the amount of time carcinogens are in contact with the wall of the bowel. However, research has suggested a more precise role for fiber. Primary bile acids are converted into secondary bile acids in the presence of anaerobic bacteria in the colon. It is the secondary bile acids, lithocholic acid and deoxycholic acid, that are promoters. When dietary fiber ferments, it produces oxygen which has an inhibiting effect on anaerobic bacteria thus decreasing production of secondary bile acids. The presence of fiber has the effect of actually reducing the amounts of lithocholic and deoxycholic acid in the bowe. Some elements in the diet are also believed to deactivate or prevent formation of certain carcinogens. For example, selenium and vitamins A, C, and E are believed to be protective in normal doses. In fact, experimental studies seem to suggest that vitamin A can actually reduce the probability that an initiated cell will become fully transformed into a cancerous cell. Obesity has been positively associated in epidemiological studies with a variety of common cancers, including endometrial, cervical, colorectal, ovarian, breast, and prostate cancer. Of these, the strongest association is between

endometrial cancer and obesity; the weakest association is with cancer of the prostate.

Use of alcohol as a beverage has long been associated with cancers of the head and neck. Alcohol is believed to affect the transport of carcinogens. Specifically, alcohol facilitates the contact between an externally introduced carcinogenic chemical and the contents of the stem cells that line the upper digestive tract and the larynx. The carcinogenic effects of cigarette smoke may be enhanced by concomitant consumption of alcohol.
Chemicals in the workplace. Since Sir Percival Pott first identified soot as a carcinogen, many other occupational chemicals have been identified and to a large extent eliminated or controlled. Working with asbestos has long been associated with cancers of the lung and with mesothelioma of the pleura and peritoneum. However, the highest incidence of cancer has been in smokers. It appears that there is a synergistic effect between asbestos and smoking. Asbestos is currently being removed from many homes and offices, and smoking has been greatly curtailed in many work settings. Cancer deaths attributable to occupational factors are believed to be decreasing, largely due to the attention paid to carcinogens in the workplace in recent years. It is estimated that more than 30% of current US cancer deaths are related to tobacco, making smoking a leading preventable cause of cancer today.
Epidemiological evidence of the carcinogenic effect of tobacco is overwhelming. In countries where smoking is prevalent, lung cancer incidence is much higher. For example, bronchogenic carcinoma is 35 times more prevalent in England than in parts of West Africa. As cigarette smoking has increased among women, lung cancer deaths have also increased. In fact, lung cancer has now surpassed breast cancer as the leading cause of cancer deaths among American women. Cigarette smoke, a complete carcinogen, is capable of both initiation and promotion.
Not only is the smoker at risk, but others sharing the smoker’s environment are also at risk. Passive smoking involves inhaling both mainstream smoke (inhaled and exhaled by the smoker) and

sidestream smoke (issues from the end of the cigarette). Many of the 50 carcinogenic substances in cigarette smoke are believed to be present in environmental tobacco smoke. Although results of studies linking passive smoking with lung cancer have been mixed, there is sufficient evidence to believe that passive smoking increases lung cancer risk.

Testing Chemical Carcinogens: Identification of carcinogenic substances is essential to cancer prevention. Two major approaches have been used to test chemical carcinogens. One involves exposure of test animals to suspected carcinogens. This type of procedure can be lengthy and is prohibitively expensive because of the large number of chemicals that need testing. Because of the time and expense involved in inducing tumors in live animals, great effort has been directed at developing screening tests using bioassay. These tests are designed to evaluate the effect of a given chemical (suspected carcinogen) on the DNA of cultured cells. To conduct the test, the suspect chemical is applied to bacteria or mammalian cells grown in culture. The goal is to see whether the test chemical or its metabolites can cause a change in the cellular DNA that is permanent (seen in both daughter cells after cell division), or a change that is not directly detectable but can cause particular cellular side effects. A number of tests have resulted, the most widely used one being the Ames test. This test has been criticized because it only identifies mutagens, leading to a simplistic approach to identification of carcinogens. Carcinogenesis is a multistep process and involves more than just mutations and often the final carcinogen has been metabolized by the body. However, the Ames test has been useful in identifying mutagens and when combined with other available information, it is of some use.

3.2 Diet and exercise

Diet, nutrition, and physical activity also rank high among the most-important determinants of human cancer risk — in part,

through their contributions to obesity, which is a known risk factor for many malignancies. Specific diet-related factors with ‘convincing’ evidence of an association with increased cancer risk, as appraised by the World Cancer Research Fund (WCRF), include, for example, aflatoxins with liver cancer, red meat and/or processed meat with colorectal cancer, alcohol with cancers of the gastrointestinal tract and, for smokers, β-carotene supplementation with lung cancer. The potential importance of diet and nutrition in cancer prevention is widely recognized, owing to an impressive amount of evidence from epidemiological, clinical, and laboratory research; however, both perceived (unsubstantiated public opinion) and real (conflicting findings in the scientific literature) inconsistencies remain. Nevertheless, guidelines on nutrition and physical activity for cancer prevention have been proposed by the WCRF and the American Cancer Society (ACS). The recommendations are consistent with those aimed at the prevention of other chronic diseases, such as diabetes and heart disease — diseases for which dietary relationships also remain under investigation. In comparison with guidelines for these other chronic diseases, however, dietary recommendations for cancer prevention are less emphasized and are not well integrated into preventive-care practice. Inconsistencies in results are common in scientific research, and often reflect an evolution in scientific thinking, but rarely are they publicized through the media to the same extent as those relating to diet–cancer relationships. As a result, confusion and scepticism surrounds the evidence linking diet and nutrition with cancer. Methodological challenges inherent in nutritional epidemiology studies in oncology have contributed to some of the inconsistent findings; such challenges have been reviewed elsewhere previously. The purpose of this Review is to provide an update on the current state of the science relating to diet–cancer relationships, and to discuss the opportunities in this area of research opened by new technologies and methodologies, in order to clarify and increase confidence in the role of nutrition in cancer aetiology.

Challenges in diet–cancer research

Evidence related to diet, nutrition, and cancer comes from many different types of research, including animal and mechanistic studies, ecological studies in humans (population-level correlation studies, which are useful for generating hypotheses), observational analytical epidemiology studies (that is, case–control and cohort studies), and trials of dietary interventions. In laboratory studies using cell cultures and experimental animal models, nutrients and other bioactive food components have been shown to affect key biological processes involved in the regulation of cell growth and carcinogenesis. Foods are naturally complex, and provide numerous bioactive substances that can act individually and/or synergistically to influence processes such as cell differentiation and apoptosis, as well as hormonal regulation of cellular functions. Demonstrating relationships between dietary factors and cancer risk in epidemiological investigations and intervention trials, however, has been challenging, owing to several broad issues. Historically, the literature on nutrition and cancer was predominantly focused on the results of case–control studies, wherein individuals with a particular malignancy (cases) recall their dietary habits before they were diagnosed with cancer, which are then compared with those recounted by healthy cancer-free individuals (controls) representative of the source population in which cancer cases arose; such studies can reveal differences that might suggest an association with cancer. Prospective cohort studies, in which healthy individuals report on their usual nutritional intake and are then followed longitudinally over time to identify individuals who eventually develop a malignancy, are increasingly represented in the current literature. The cohort design reduces the risk of recall bias, which can lead to error in the measurement of dietary intake, and establishes a temporal sequence, as dietary data are collected before cancer diagnosis. Patients with certain types of cancers (for example, gastrointestinal tumours) could potentially have symptoms that lead to dietary changes before diagnosis, which might lead to

erroneous diet–cancer associations (reverse causation); however, this can be minimized by excluding from the analysis patients whose dietary data were obtained in the immediate time period (1–4 years, for example) before cancer diagnosis. Cohort-study designs are also less prone to biases related to selection of participants, compared with case–control designs; selection bias can lead to a study population that is not representative of the population of interest, for example, with declining case–control study participation rates in the past decades, especially among control subjects. Nonetheless, cohort studies also have limitations, including the need for very large sample sizes and long follow-up durations to capture the development of disease. The large sample size increases the costs associated with collection of detailed data. Furthermore, case–control studies with robust measurement methods can be more useful for investigations of rare cancers or certain subgroups, as accrual of the appropriate numbers of participants to a cohort study would be difficult; the shorter study durations and smaller sample sizes of case–control studies also allow for more comprehensive and targeted data collection. Randomized controlled trials (RCTs) of dietary changes or supplements have long been considered to produce the highest level of evidence on diet–cancer relationships, which is appropriate for causal inference (that is, to establish that a given dietary factor is causally associated with cancer). By design, randomization controls for confounding factors related to both diet and the cancer of interest that could distort measures of association of the diet–cancer relationship. In cohort studies, these confounding factors can be controlled for in the analysis phase, but such studies remain prone to unmeasured confounding variables, or inadequate measurement of and control for the confounding factor. Intervention trials have important limitations, however. In trials of behavioural interventions, both adherence to the intervention and the intervention–outcome relationship are tested. Nonadherence to the protocol, or an insufficient magnitude of change in diet, will affect the likelihood of detecting change in the risk of a cancer

outcome; that is, the actual difference in nutritional intake in the intervention versus control groups might be insufficient to achieve a meaningful difference in the dosage of the bioactive food components hypothesized to affect carcinogenesis. Furthermore, dietary effects probably differ based on baseline nutritional status. Of note, participants who volunteer for trials of dietary interventions tend to be more health-conscious, and typically have reasonably good dietary intakes at baseline assessments, as compared with the general population. For example, in a large study to test the effect of a diet high in vegetables and fruits on breast-cancer recurrence, as an intervention, the participants were consuming an average of more than seven servings per day at enrolment, which is considerably higher than is typical for women in the general population. Moreover, a growing body of evidence indicates that much of the benefit observed for dietary or nutritional interventions is attributable to improvements in the dietary status of people who were previously nutritionally inadequate. Thus, interpretation of the findings of negative trials (those in which no benefit is observed) is difficult if the study was conducted in a well-nourished population. Dietary or nutritional-supplement intervention trials are conducted over a few years, which is a brief period relative to the long latency of most cancers, and this fact is an important issue in interpreting results. Depending on the proposed mechanism of action, the timing of the intervention might affect the outcome; for example, the intervention might be applied too late in the cancer continuum if the individuals are at a very high risk of the disease, or already have preneoplastic lesions or cellular changes that cannot be reversed. Furthermore, dietary effects are likely to be manifest only in the long-term, thus limiting the feasibility of conducting RCTs —owing to the need for large sample sizes, and major investment of time and resources.

Dietary data collection methods

Self-reported dietary data (with or without complementary biospecimens) provide the foundation for diet– cancer research.

Food frequency questionnaires (FFQs) have been almost universally relied upon to obtain such data. FFQs are used to inquire about the usual frequency of consumption (with or without consideration of portion size) of a limited list of food items over a designated time frame (usually 12 months). Being self-administered and machine-readable, FFQs are considered easy to use, with practical application to large study cohorts. Seasonality in dietary habits can also be considered, as FFQs enable assessment of diet over a long timeframe. Investigators can use FFQs to rank respondents with respect to their level of food intake; however, the resulting data are not quantifiably precise because they relate to only a subset of foods, and with limited emphasis on assessing portion-sizes consumed. Furthermore, poor recall or a desire to be socially acceptable can contribute to measurement inaccuracies in self-reported foodconsumption data. A number of assumptions made when assigning nutrient values to foods (for example, assuming all foods in a class, such as breads or yoghurt, have similar nutrient levels) might also introduce measurement error of nutrient intake. In methodological research aimed at improving the accuracy of dietary assessment, the data obtained using different self-report methods have been compared (FFQs versus 24-h recall), and measurement errors were shown to be correlated — that is, participants similarly under-report food intake by both methods. Assessment of biomarkers in blood and urine samples can enable objective validation of dietary intake, and food-consumption estimates from self-reported questionnaires can be calibrated using equations derived from linear regression of biomarker values on intake estimates. Validation or calibration studies can be undertaken on a smaller subset of participants within the overall study populations, thus limiting costs. Two ‘recovery’ biomarkers have been used increasingly in such studies: doubly labelled water (DLW), a tool used to objectively estimate energy expenditure; and urinary nitrogen excretion (a biomarker of protein intake) — although the use of DLW stable isotopes is cost-prohibitive for assessment

in most epidemiological studies. Notwithstanding, the US government invested in an important large-scale methodological validation study, the Observing Protein and Energy Nutrition (OPEN) study , in which these two biomarkers were used to assess the degree of measurement error associated with the FFQ versus a short-term (24-h) recall method in healthy men (n = 261) and women (n =223) aged years. The results demonstrated that, in the short-term, energy and protein intake were substantially underestimated when the FFQ was used, attenuating relative-risk estimates by up to 50%. In fact, biomarker-based data showed under-reporting of both protein and energy intake when using either the FFQ or 24-h recall method; individuals with a body-mass index (BMI) indicative of obesity were found to have the greatest degree of under-reporting. Differential under-estimation of energy intake among overweight or obese persons might distort the direction of diet–cancer associations. Notably, the protein to energy ratio calculated from the two self-reported dietary-assessment methods was similar to that indicated by the objective biomarker measurements. Thus, nutrient intakes are commonly adjusted according to reported total energy intake, in order to reduce the problem of differential reporting of overall food-energy intake by obesity status. Calibration of FFQ data using recovery biomarkers has similarly been applied in large epidemiological studies, such as the Women's Health Initiative (WHI). Using biomarker calibration, statistically significant associations between energy intake and the risk of invasive cancer, and between fat density and the risk of postmenopausal breast cancer have been demonstrated — these relationships were not identified previously using food-intake data alone. In the calibrated analysis, controlling for BMI reduced the strength of the association between energy intake and the risk of cancer, suggesting that changes in adiposity probably underlie this relationship; by contrast, adjustment for BMI strengthened the results for fat density measured by 4-day food record and calibrated using energy biomarkers. Thus, consideration of

objective dietary biomarkers has forced the recognition that the most-convenient method to assess diet is not especially accurate, but also that ‘correcting’ estimated nutrient or food intake based on projected energy intake (that is, using nutrient to energy ratios) has the potential to reduce inaccuracies and uncover previously unidentified diet–cancer associations.

To date, the limited number of recovery biomarkers available has constrained the conduct of calibration studies in nutritional epidemiology investigations. In addition to DLW and urinary nitrogen, urinary potassium and sodium have been used in this context. Additionally, concentration biomarkers that are correlated with intake of a food or food group, but are not present in quantities directly proportional to overall intake, are available — such as carotenoids as a biomarker for fruit and vegetable intake. To obtain unbiased estimates of intake of a food or food group, however, the biomarker itself must be calibrated to true intake in a ‘feeding study’, in which feeding is administered and monitored in a controlled setting, and the biomarker must be measured for all individuals in a study, rather than a smaller subgroup.

In addition to the use of biomarkers for ‘calibration’ of self-reported dietary data, data collection in epidemiological studies of nutrition is now performed using multiple dietary-assessment instruments, such as FFQs together with recall or diary-based methods. For example, prospective food records were shown to enable better estimation of energy and protein intake, compared with retrospective FFQ data, whereas the validity of 24-h recall data was moderate in comparison with biomarker data in the WHI. Furthermore, factoring BMI and age — which are routinely included in statistical models of diet–disease relationships in epidemiological studies — as well as ethnicity into calibration equations can substantially improve the accuracy of food-intake estimation. Data pooled from five large observational studies, in which dietary intake was measured, demonstrate that multiple (two or more) 24-h recalls measured diet with greater validity

than that achieved with a single measurement, and reinforced that BMI was a strong predictor of under-reporting. A more-recent analysis of data from the Eating at America's Table Study found the optimal approach for minimizing reporting errors in dietary assessments was at least four 24-h recalls plus a single FFQ. An important feasibility issue for all dietary-assessment tools (including biomarkers), however, is the timing of measurement. For some outcomes, the window of exposure that is relevant to development of disease (which might be during gestation or childhood, for example) might not be captured appropriately with measurement of nutritional status in adulthood.

The recognition that 24-h-recall data adds value to the validity of dietary intake estimates has encouraged the development of innovative online monitoring methods, such as the Automated Self-Administered 24-Hour Dietary Recall (ASA24) instrument. This recall instrument is available now and can be feasibly incorporated into studies for data collection many times over different time periods. Audio and imaging capture of food via mobile telephone, electronic food records, computerlinked food weighing, and food-package-barcode scanning could eventually replace paper questionnaires for dietary-intake estimation.

Reductionist versus holistic concept of diet

Older studies, as evidenced by the content of the 'Diet and Health' report, had a primary emphasis on nutrients (usually single or groups of related micronutrients) and their effects on chronic disease risk, reflecting the reductionist scientific point of view that prevailed at the time. The focus on particular nutrients was attractive, as any associations identified, if real, could lead to highly feasible preventive **interventions,** such as food fortification and supplementation. The emphasis on micronutrients continued until the mid-1990s, when the results of many large and highly publicized trials failed to reveal a benefit and, in some cases, demonstrated harm from dietary supplementation with micronutrients for the prevention of chronic diseases (including cancer). These findings prompted

recommendations from the US Preventive Services Task Force to avoid vitamin and mineral supplementation for the primary prevention of cancer and cardiovascular disease. Epidemiological evidence relating to dietary factors can be challenging to interpret because food and eating patterns are inherently complex and multifacteted. For example, a diet high in vegetables and fruits provides numerous potentially beneficial constituents, such as folate, polyphenols, carotenoids, and fibre; therefore, focusing on a specific nutrient might result in misleading findings. Even a focus on single foods or food groups (for example, grapefruit) should be cautiously applied in the absence of substantial supporting scientific evidence, because foods are consumed as part of an overall dietary pattern that comprises many variables — including differential amounts of healthy and unhealthy constituents, and dietary factors. Furthermore, lifestyle behaviours, such as diet quality, physical activity, weight management, and smoking status, are typically clustered; thus, teasing out the effect of a single element or behaviour is difficult.

For ‘macronutrients’ especially, the emphasis on a broad category of nutrients (such as fat), rather than their individual components and food sources (for example, saturated fatty acids from animal products versus monounsaturated and polyunsaturated fat from plant sources or fish) has introduced complexities in the study of diet–cancer relationships, and arguably contributed to inconsistencies in findings. As an example, the hypothesis that total fat intake is associated with breast cancer stemmed from ecological data on fat intake and breast-cancer mortality in 23 countries, along with supporting experimental data from animal studies, as reviewed by Wynder and colleagues. Subsequent findings from some studies were null, whereas other studies reported a positive association. Moreover, two large intervention trials on the effect of a low-fat diet found conflicting results for the association of fat intake with breast-cancer recurrence, and a borderline reduction in risk of first breast cancer was demonstrated in another trial of a low-fat

dietary intervention. Reductions in the intake of any energy-containing nutrient, such as ‘fat’, are associated with variability in both the particular types of fat for which intake is reduced, as well as other energycontaining nutrients that replace the lost calories. That is, a decreased proportion of dietary saturated fat could be offset by higher consumption of sugars or starches, or other types of fat, and each of these diets are unlikely to have similar effects on cancer prevention. The ultimate translation of a broad dietary recommendation to reduce fat intake is, therefore, variable, subject to interpretation, and as has become apparent with time, an ineffective strategy for reducing chronic-disease risk.

Thus, a focus on dietary patterns is a more useful strategy for cancer prevention. In the past decade, systematic reviews have summarized the data pertaining to dietary patterns and oesophageal squamous-cell carcinoma, breast cancer, gastric cancer, and colorectal cancer, and the body of literature on dietary patterns in relation to cancer continues to grow. Measuring the association between dietary patterns and cancer-related outcomes enables consideration of the fact that intake of many foods is correlated and that these foods might interact to affect cancer risk. Dietary-pattern analysis can be agnostic, using statistical clustering techniques, or a priori, based on recommended patterns of eating, such as the Healthy Eating Index (HEI) — a measure of diet quality. These approaches have been compared for their associations with colorectal-cancer risk in the National Institutes of Health (NIH)–American Association of Retired Persons (AARP) Diet and Health Study. Each approach addresses a slightly different question: cluster analysis can be used to group together the people in a population who have a similar dietary intake, and provides information on what represents the typical diet for these groups (for example, ‘many foods’, ‘vegetables and fruits’, ‘fatty meats’, ‘fat-reduced foods’, ‘diet foods/lean meats’); factor/principal component analysis (also data-driven) is used to identify foods that are correlated with regard to diet, and suggests explanatory factors that

underlie population dietary trends (for instance, ‘fruits and vegetables’, ‘reduced-fat/diet foods’, ‘meat and potatoes’; and ‘index’ analyses (a priori) score individuals with regards to their overall diet based on predefined criteria (such as the HEI, the Alternative Healthy Eating Index, the Mediterranean Diet Score, or the Recommended Food Score). New research suggests that analyses of dietary patterns might enable relationships between cancers and diet that have been only weakly associated using more-conventional approaches (nutrient or food-based exposures) to be better discerned. For example, the WCRF/AICR Continuous Update Project did not identify convincing evidence for a link between any single food or nutrient and pancreatic-cancer risk, whereas a large analysis of the NIH–AARP Diet and Health Study revealed that consuming a diet ranked in the highest quintile of the HEI-2005, compared with the lowest quintile, was associated with a substantially lower risk of developing pancreatic cancer. Lifestyle-based indices that rank individuals according to adherence to cancer-prevention guidelines (incorporating both diet and physical activity) have also identified associations between better compliance with these guidelines and lower risks of developing many types of cancer.

Dose–response relationships

Another important way in which the scientific evidence for associations between diet and cancer has evolved is that older studies often assumed linear dose–response relationships, and data were analyzed based on this assumption. By contrast, current studies take advantage of more-sophisticated analytical approaches that model the ‘best fit’ of relationships according to the **data** (for example, using restricted cubic spline regression), rather than assuming linearity. A growing body of literature relating to the use of this more-modern approach has indicated that having a very poor status for a variety of different nutrients is associated with a higher risk of malignancy; however, having a very high (that is, supraphysiological) nutrient status is similarly and paradoxically associated with excess risk — indicative of a so-called ‘U-shaped curve’ relationship. Of note, very large

studies or meta-analysis might be required to have sufficient sample size to appropriately model this U-shaped relationship between dietary factors and cancer. In a meta-analysis of prospective studies, investigators modelled the relationship between intake of fruits and vegetables (combined and separately), and colorectal-cancer incidence according to linear and nonlinear dose–response, in order to examine the shape of the relationship. The investigators argued that inappropriate statistical modelling of the association as linear might have driven the null findings in previous studies. Failure to appreciate a nonlinear relationship has also resulted in null associations between micronutrient intake and cancer risk in both observational studies and clinical trials, as has been discussed elsewhere. The issue of nonlinearity might be exaggerated in RCTs of micronutrient supplements, in which the intake of certain nutrients is added to the usual intake from food; as previously highlighted, volunteers for dietary intervention studies are more likely to have overall healthier diets, potentially compounding this issue.

Tumour heterogeneity

The biology of cancers arising within and across different organ sites is known to be heterogeneous, and this variability is another issue that affects the interpretation of research on the role of dietary factors in cancer risk and disease progression. A clinically-detected cancer can arise via a number of carcinogenesis pathways, through various genetic mutations; therefore, tumours originating within the same organ site are often aetiologically very different. Characterization of aetiological subtypes, as well as clinical subtype (with common prognostic characteristics, such as treatment response) is driving a move towards personalized cancer therapy, as well as informing diet–cancer research efforts. For example, squamous-cell carcinoma of the oesophagus is aetiologically quite different from adenocarcinoma of the oesophagus: the former is more-strongly linked with smoking and drinking alcohol in Western populations, or human papillomavirus (HPV) infection in other

populations, such as those in China; the latter is more-closely associated with obesity and/or reflux disease. Furthermore, the dietary factors associated with these two cancers have some overlap, but are also quite distinct. Similarly, an analysis from the NIH-AARP Diet and Health Study highlighted that the elevated risk of endometrial cancer among obese compared with non-obese women was greater for type I (endometrioid) compared with type II (non-endometrioid) tumour subtypes. With respect to nutrients, higher intake of phytoestrogens was associated with a reduced risk of mucinous ovarian cancer, but not other ovarian cancer subtypes, in the Australian Ovarian Cancer Study. Another example of the heterogeneity in diet–cancer associations according to cancer subtype comes from patients with breast cancer. Research has revealed up to 21 distinct subtypes of breast cancer with different risk factors, aetiologies, and outcomes; dietary associations also vary by tumour subtype. For example, in the large, long-term Pooling Project of Prospective Studies of Diet and Cancer, a protective effect of fruit and vegetable intake on oestrogen receptor (ER)-negative breast cancer, but not on ER-positive breast cancer, was consistently demonstrated across 20 studies. Thus, the somewhat different conclusions as to the role of various nutritional factors in disease aetiology, based on data from earlier studies that failed to account for differences by histology, are perhaps not surprising. Increasing recognition that cancers at a given organ site are a collection of aetiologically distinct subtypes is improving aetiological research, and is stimulating the development of more-effective therapies that are personalized to tumour characteristics. Future studies are needed, in which each cancer is considered as pathologically and genetically distinct, and that are powered for subtype analyses.

Evolution of dietary recommendations

The volume of research, types of data available, methodologies used, and analyses and interpretations of the literature on the relationships between food, nutrition, and cancer has increased substantially over the past few decades. Nevertheless,

examination of the recommendations that have been issued by authoritative groups (see the ‘Historical perspective’ section) reveals that dietary guidance has remained very consistent over the years, in contrast to the results of individual studies of diet–cancer relationships, which are often inconsistent. Thus, despite the enormous change in the scientific evidence in this field, overall, the primary conclusions and public guidance have remained remarkably similar, at least if one considers only the guidance issued by authoritative bodies. In both the first WCRF/AICR report published in 1997 and the second report published in 2007, the following recommendations were made: maintain a body weight within the lower end of the normal range (BMI 21–23 kg/m2); be physically active (equivalent of 60min of exercise per day); consume a plant-based diet comprising a variety of fruits and vegetables; choose unprocessed cereals and grains over processed products; limit intake of red meat and salt; avoid or limit alcohol consumption; avoid mouldy grains or mouldy legumes; and meet nutritional needs without the use of dietary supplements. In the first report, the WCRF/AICR recommended that total fat should only provide 15–30% of the total daily calories consumed, with advice to limit consumption of fatty foods, particularly those of animal origin, whereas in the second report , they specifically emphasized avoidance of energy-dense foods, sugary drinks, ‘fast foods’, and processed meat, and did not provide a specific dietary fat
recommendation — reflecting a shift from a nutrient-centric approach to a more food-based approach. The scientific evidence that micronutrient supplementation represents a viable strategy for cancer prevention in the general population has eroded considerably over the past several decades; however, authoritative bodies did not previously recommend micronutrient supplements for cancer prevention. The perceived inconsistencies regarding this strategy are largely attributable to three factors: widespread promotion of micronutrient supplements or individual food products by for-profit entities; the results of individual studies that go against the prevailing

wisdom being preferentially picked up and disseminated by media outlets eager to stoke controversy; and promotion of individual studies or findings by scientific authors and/or their institutions for purposes of self-promotional recognition. On this basis, we argue that the stability of many dietary recommendations for cancer prevention over the past 20–30 years is actually quite remarkable, with perceived inconsistencies reflecting failures in communications rather than failures in the processes driving scientific policy.

3.3 Infection

It is almost 40 years since Barry Marshal and Robin Warrens identified and recovered Helicobacter pylori (H. pylori) from gastric biopsy specimens. They later demonstrated the role of H. pylori in peptic ulcers, and in 2005, they were awarded the Nobel Prize in medicine for these groundbreaking discoveries. Later, it became clear that H. pylori has a carcinogenic role and the fact that a course of antibiotics influenced cancer occurrence was truly a 'game changer'. During the following decades, the examples of associations between infections and cancer were constantly growing. Currently, it is estimated that infections play a role in around 16% of all cancers worldwide and this number may well grow with increasing research in the field. A now well-known example is the association between certain human papillomavirus (HPV) types and cervical cancer among women, ano-rectal cancer among males and naso-pharyngeal cancer. In these cases, vaccine research and subsequent vaccine implementation may reduce, and even in some cases, eliminate, the prevalence of severe and debilitating cancers. The association is not limited to bacteria or viruses but also exist between parasitic infections and certain cancer types: Schistosoma haematobium and bladder cancer, Chlonorchis sinensis and cholangiocarcinoma mentioned are examples. In this issue of APMIS, we have invited researchers from various disciplines to give a state-of-the-art review of their research field

within different infectious organisms and describe the association to cancer. In many cases, we are still lacking an in-depth understanding of the pathogenetic mechanisms, essential to broaden our understanding of the term ‘carcinogenesis’ and to develop improved treatment regimens.

Historical Review: The relationship between viruses and cancer in animals was first recognized in 1911 when a farmer brought a chicken suffering from an unusual growth to Dr. Peyton ROUS, a researcher. The chicken had a sarcoma caused by what later came to be known as the Rous sarcoma virus. Since that first discovery, scientists have uncovered relationships between various viruses and feline and bovine leukemia, lymphoma in chickens, papilloma in rabbits, and others. Although the relationship between viruses and various cancers in animals has been recognized for many years, the relationship between viruses and human cancer is a fairly recent discovery. And to date, only a few cancer causing viruses have been identified in humans.
Viruses work by affecting DNA and causing mutations. In some cases, the virus becomes integrated into the chromosomes of the normal cell; in others, it alters chromosome structure. The net result is activation of oncogenes and inactivation of cancer suppressor genes. Hepatitis B virus. Epidemiological data support the relationship between the hepatitis B virus (HBV) and hepatocellular carcinoma. For example, cancer of the liver is 100 times more prevalent in southeast Africa, where HBV is endemic, than in England. The incidence of liver cancer among black Americans is relatively low, similar to white Americans. HBV is also endemic in Asia, where the incidence of hepatocellular carcinoma is also very high.
Human T-cell leukemia-lymphoma virus. Human T-cell leukemia-lymphoma virus (HTLV- 1) is a retrovirus thought to be responsible for some T-cell leukemias in adults. HTLV-1 is endemic in some parts of Japan, in areas of the Caribbean and Africa, and in the southeastern United States. The virus, which appears to be transmitted by either sexual contact or

contaminated blood, is known to have a latency period from several years to 40 years. HTLV-2 has been isolated in the T-cell variety of hairy cell leukemia. However, hairy cell leukemia is a disease of the B lymphocyte with relatively few cases affecting T lymphocytes. The Epstein-Burr virus. The Epstein-Barr virus (EBV), which is endemic in parts of the African continent, is a DNA-type virus of the herpes family. It stimulates B lymphocytes to proliferate. When the host immune system is intact, the T cells control the proliferating B cells. However, when the immune system is dysfunctional following a disease (ie, malaria, the acquired immunodeficiency syndrome [AIDS], or cyclosporin administration), the T cells may lose the battle and a neoplasm such as Burkitt's lymphoma may result. In the United States, Burkitt's lymphoma is relatively rare. EBV is associated with the benign disease infectious mononucleosis. Hodgkin's disease has been linked with EBV in the United States but the data are conflicting. In China, EBV is believed to be a causative factor in the high incidence of nasopharyngeal carcinomas.
The human immunodeficiency virus. The human immunodeficiency virus (HIV) is a retrovirus that causes the condition that has become known as AIDS. Although not an oncogenic virus per se, HIV, through its suppression of the immune response, results in the development of opportunistic tumors such as Kaposi's sarcoma. There are five known routes of transmission of HIV: sexual contact, needle sharing, exposure to blood or blood products, through breast milk, and through the placenta while in utero. It is estimated that millions of individuals worldwide are infected with HIV but are, as yet, asymptomatic. Thus, although many oncology nurses have never seen Karposi's sarcoma, its incidence may be expected to increase as more cases of AIDS are confirmed. Human papilloma viruses. Human papilloma viruses (HPV) affect squamous epithelium. This is the family of viruses that is responsible for common warts seen on the skin and genital warts, known as condylomas. DNA from HPV-16 and HPV-18 may be found in 70% of all cervical carcinomas. Although the association between HPV and cervical

cancer is strong, the associations between HPV and cancer of the penis and prostate are less definitive.
Herpes simplex virus type II. Herpes simplex virus type II (HSV) is also sexually transmitted and has also been implicated in cervical cancer. For decades, sexual promiscuity was linked in epidemiological studies with cancer of the cervix. Today, the association is believed to be due to the sexual transmission of two oncogenic viruses, HPV and HSV. Current research suggests that multiple factors may account for the occurrence of cervical carcinoma and that HSV-II and HPV may actually be synergistic in causing the development of this cancer.

3.4 Radiation

Although there is no longer room for doubt that ionizing radiation may cause cancer in man, any estimate of the potential danger, in this regard, of increase in the level of environmental radiation contains an element of uncertainty. This arises from our lack of information regarding the shape of the curve relating dose rate (incident radiant flux) and the incidence of cancer in the population. Without such information we are forced, in any estimation we may make, tacitly to assume a shape for that curve, and in this we are likely to be influenced by our ideas of the mechanism of carcinogenesis, a matter on which there is not general agreement. The assumption of a linear relationship may be justified as a first approximation, but this does not have experimental or other support. This article is presented with the hope that data relating to another kind of environmental radiation -ultraviolet light-may be of service in this regard.
Induction of Cancer by Ultraviolet Light "Non-ionizing" radiation in the ultraviolet spectrum induces cancers of the skin of experimental animals with quantitative predictability, the longwavelength limit for this carcinogenic action lying at about 0.32 μ. Sunlight, having its short-wavelength limit at about 0.29 μ, contains a small fraction of these carcinogenic wavelengths, and evidence converges to indicate that this is a principal cause

of cancer of the skin in man. The evidence includes, besides inferences from the experiments on animals, the fact that skin cancers are largely limited to exposed areas--principally the face- and that there is a correlation with latitude such as might be expected if this radiation were the cause. The carcinogenic wavelengths are the same as those that cause sunburn, and it is probable that there is at least an indirect causal relationship between the two processes. The outer horny layer of the skin acts as a protective filter against the sunburnproducing, carcinogenic, radiation, and this is found to be more opaque for negro than for white skin. Correspondingly, skin cancer is relatively rare in the Negro population as compared to the white. Cancer induction by ultraviolet radiation has been the subject of a good deal of quantitative experiment, and it would seem reasonable to attempt to apply some of the information gained therefrom to the case of ionizing radiation-- lacking as we do the comparable information regarding the latter. I have discussed elsewhere various aspects of carcinogenesis by ultraviolet light; not all of these aspects will be treated here, but only those that seem to bear directly on the present problem. For this purpose, it will be unnecessary to adopt any specific hypothesis for the intimate mechanism, although the quantitative evidence regarding carcinogenesis by ultraviolet light appears to exclude some of those that have been proposed.

After Rontgen discovered X-rays in 1895, it was recognized that radiation exposure causes acute tissue damage. Later, it was found out that cancer, particularly leukemia, is induced by exposure to radiation. By the early 1970s, accumulated evidences demonstrated that radiation is capable of inducing cancer in many types of tissues. It became possible to estimate the risk of leukemia and solid cancer primarily on the basis of the data collected from the survivors of the 1945 atomic bombings in Hiroshima and Nagasaki. During the 1980s, the data from the follow-up of A-bomb survivors provided revision of the earlier risk estimates. However, since the risk estimates have been obtained from epidemiological studies of A-bomb survivors, they

are appropriate for populations at high doses. Thus, a reducing factor of 2, which is called a dose and dose-rate effectiveness factor (DDREF), has been proposed for exposure at low doses or at low dose rate, while another report proposed a DDREF value of 1.5. Further information from a number of epidemiological studies of cancer induction by exposure to external and internally incorporated radioactive nuclides has indicated that caution is needed in interpreting the dose – response relationships obtained by direct extrapolation from epidemiological studies conducted in A-bomb survivors, particularly at lower doses of ,100 mSv of low-linear energy transfer (LET) radiation. In the following sections, every aspect with the emphasis on low-dose radiation effects will be taken into consideration. Particularly, much attention has been paid to the dose – response relationship between radiation doses to the thyroid gland and thyroid cancer incidence. Specific genetic alterations found in papillary-type childhood thyroid cancers after Chernobyl accident and their possible relation to radiation signature will also be discussed.

PHYSICAL EFFECTS OF LOW-DOSE RADIATION

DEFINITION OF LOW-DOSE RADIATION: Based upon the dose – response for mortality from solid cancers among A-bomb survivors, United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) 1993 report considered that a low dose could be ˂200 mGy. Lately, it was reported that statistically significant risk elevation is not observed at doses of 100 mSv or less, so that a low dose could be 100 mSv or less. The Biological Effects of Ionizing Radiation (BEIR) VII report of the US National Academy of Sciences defined low dose as doses up to ~100 mSv. In this review, low doses are defined as those ~100 mSv.

UNITS OF RADIATION EXPOSURE: The quantity used to refer to the amount of ionizing radiation is absorbed dose, which is defined as the energy absorbed per unit mass. The unit of absorbed dose is gray (Gy), and 1 Gy equals 1 joule of energy absorbed per kilogram of matter. As different types of radiation produce different biological effects, equivalent dose, which is the

product of absorbed dose and radiation-weighting factor, is introduced. The unit of equivalent dose is sievert (Sv). Finally, effective dose is used to limit health risks involved in radiation exposure. Effective dose is the sum of all of the weighted equivalent doses in all the tissues and organs exposed. Since different tissues have different radiation sensitivities, tissue-weighting factors are used to calculate weighted equivalent doses. Thus, if the effective dose is used for radiation exposure, radiation health effects are the same between external and internal exposure. The unit of effective dose is also sievert (Sv). DIRECT AND INDIRECT EFFECTS: Absorption of radiation energy to DNA directly induces structural alterations of DNA, which is called a direct effect. Alternatively, interaction of radiation with water molecules in the cell produces water-derived free radicals that indirectly cause DNA damage. This action is called an indirect effect. It is estimated that low-LET radiation with 100 mGy causes at least 100 instances of oxidative DNA damage, ~100 DNA single-strand breaks (SSBs) and ~4 DNA doublestrand breaks (DSBs). For low-LET radiation, 60 – 70% of such DNA damage is estimated to result from indirect effects, while 30– 40% of the damage is caused by the direct effect. Although free radicals are created along the radiation track, radiation is not the only source to generate them. It is well known that endogenous (intracellular) free radicals, which are collectively called reactive oxygen species (ROS), arise from mitochondrial oxidative metabolism and other reactions in cells. The estimated average generation rate is ~10^9 ROS per cell per day, which results in 10^6 oxidative DNA damage, 10^5 SSBs and 0.1 DSBs per cell per day. The rate is much higher than those estimated in cells receiving 100 mGy per year, which is ~0.01 DSBs per cell per day. Although it was not described more in detail, such low-dose rate exposure should be treated as totally different from high-dose rate or acute exposure. For example, acute 100 mGy of radiation induces four DSBs per cell at once, while 100 mGy per year creates approximately one DSB in a cell out of 2400 cells/h.

CELLULAR EFFECTS OF LOW-DOSE RADIATION

DNA DAMAGE AND REPAIR: Both direct and indirect absorption of radiation energy to genetic materials results in structural alterations of DNA. A variety of changes—so-called DNA damage—have been identified. Those include base damage, apyrimidinic/apurinic (AP) sites, SSBs, DSBs and cross-links. The yield of DSBs has been calculated as described earlier, and ~40 DSBs could be induced per gray. Theoretical numbers of DSBs have been confirmed experimentally by counting the foci formation of DNA damage checkpoint factors, and ~40 foci are reported to be induced by 1 Gy of low-LET radiation. Induction of DNA damage by low-dose radiation has been quantified by foci formation, and over a few mGy to 1000 mGy dose range, the induction shows a linear dose – response. In vivo formation of DSBs was also examined in lymphocytes obtained from individuals undergoing computed tomography examinations. It was found that the number of DSBs increased linearly depending on the dose – length product.

The ability to repair DNA damage is inherited through evolution. Most of the repair systems found in prokaryotes exist in mammalian cells. Thus, oxidative DNA damage, such as base damage, AP sites and SSBs, is efficiently repaired through the base excision repair and SSB repair pathways. The first step in base excision repair is the excision of modified base, which is catalyzed by DNA glycosylase. The resultant AP sites are cleaved by AP endonuclease, which result in SSBs. Nucleotides gaps are filled with DNA polymerases, and DNA termini are rejoined by DNA ligases. Oxidative base damage, such as 8-oxoguanine, causes mismatch base pairing during DNA replication and eventually induces mutation. AP sites as well as SSBs have also been considered pre-mutagenic lesions. DNA DSBs result in disruption of the higher-order structure of chromatin, which manifests itself as chromosomal aberrations. Multiple DNA repair pathways are involved in repairing DSBs. Non-homologous end joining (NHEJ) is the major repair pathway for DSBs through the cell cycle. Classical NHEJ does not use any sequence

homology; therefore, it does not need DNA end processing. However, alternative-NHEJ and homologous recombination (HR) are dependent on DNA end resection. HR is functional only after the sister chromatid is provided through DNA replication. In principle, HR is an error-free pathway, whereas NHEJ, particularly alternative-NHEJ, is error prone.

Since DSBs are spontaneously generated during DNA replication, or produced by specific nucleases during V(D)J recombination, class switch recombination and meiotic recombination, in addition to those induced by endogenous ROS, repair of DNA DSBs has been an efficient process, and reparable DSBs are generally eliminated within 24 h of radiation exposure. In fact, it has been shown that DSBs induced by X-ray doses of up to 200 mGy are completely repaired in proliferating human cells after 24 h. Thus, while the initial induction of DSBs shows a linear dose – response relationship, DNA damage caused by low-dose radiation has little chance to persist in cells. This is in sharp contrast to those induced by high-dose radiation, which often result in residual DSBs. Repair DSBs were also examined during chronic low-dose-rate irradiation. In vitro experiments have shown that normal human diploid fibroblasts exposed to g-rays at a dose rate of 18 mGy/h do not accumulate DSBs nor phosphorylation of p53. According to a previous report, endogenous DSBs are formed from single-strand DNA lesions, including SSBs, AP sites and oxidative base damage, in replicating cells at a rate equivalent to that of DSBs induced by radiation at a dose rate of 282 mGy/h (33). Therefore, around this dose rate, human cells are expected to repair DSBs efficiently and faithfully. This assumption is in good agreement with the data showing that DSBs induced at a dose rate of 238 mGy/h are repaired with no error. Although increased levels of residual DSBs were observed with 102 mGy/h in confluent non-dividing cells, a similar paper has reported that DSBs induced in quiescent normal human fibroblasts by very low-dose radiation, such as 1 mGy of X-rays, remains unrepaired for many days, but it is rapidly repaired if the cells are allowed to proliferate. Thus,

it is obvious that low-level DSBs are efficiently repaired with high fidelity especially in proliferating human cells.

DNA DAMAGE RESPONSE: While DNA repair pathways amend DSBs efficiently, a certain fraction of the initial breaks possibly remains unrepaired. Such lesions could be complex lesions or clustered damage, or DSBs induced in heterochromatic regions. If cells with residual DSBs are replicated, the stability of the genome is threatened. Thus, cells have evolved a system called DNA damage checkpoint, by which the integrity of the genome is maintained. The central players of DNA damage checkpoint pathway are ataxia-telangiectasia mutated (ATM) and p53 proteins. Once DSBs were sensed by ATM, it is activated as a protein kinase and catalyzes phosphorylation of downstream factors, including p53. The p53 protein is a well-known tumor suppressor and regulates transcription of various genes, whose products regulate cell death or irreversible growth arrest. Recently, it has been shown that DNA damage signal is amplified through formation of a multiple protein complex. ATM-dependent phosphorylation of a histone H2AX, a member of histone H2A, initiates sequential protein interactions, and phosphorylation of histone H2AX expands for over several mega base chromatin regions surrounding the initial DSB site. Thus, these protein complexes come to be visualized as discrete foci under a fluorescence microscope. Moreover, the number of foci is well correlated with the actual number of DSBs, so that the foci are now widely used as sensitive surrogate markers for DSBs. Amplification of DNA damage signal plays a crucial role when the number of instances of DNA damage is small. It is essential for execution of cell cycle arrest, particularly G1 arrest, as AT cells defective in ATM function fail to initiate G1 arrest. Although a recent study reported that the G1 checkpoint was inefficiently maintained, it should be mentioned that cells with DNA damage terminate cell proliferation at the G1 phase within a next few cell cycles. Thus, cells have evolved a sophisticated system by which they can respond to very limited number of DSBs induced by low-dose radiation.

LOW-DOSE RADIATION AND CARCINOGENESIS

A-BOMB SURVIVORS: The most informative epidemiological study of the survivors of the atomic bombings at Hiroshima and Nagasaki has been conducted by the Radiation Effects Research Foundation (RERF). The Life Span Study (LSS) is based upon large numbers of persons with various whole-body doses. Excess relative risk (ERR), which is a measure of the size of the increase in cancer risk in the study population due to the radiation at given doses, has been used to examine the relationship between radiation doses and the risk of cancer induction. The latest report on LSS mortality from RERF demonstrated that the dose – response relationship at low doses below 1 Gy might be described by both a linear and a curvilinear function. The ERR estimate for solid cancers was 0.47/Gy. In the dose range 0 – 150 mSv, the excess risk of solid cancer seems to be linear; however, there is no statistically significant elevation in risk at doses below 100 mSv. The strong link between radiation exposure and thyroid cancer was also provided by studies of A-bomb survivors. The dose – response relationship appeared to be linear, and the gender-averaged ERR estimate was 0.57/Gy. Age at exposure is the most important modifier of thyroid cancer risk, and elevated risk is no longer detectable among survivors exposed after the age of 30. The A-bomb survivors received higher external doses over a short period, which is in contrast to the other populations receiving low-dose radiation over long periods. However, as the most-esteemed epidemiological study of radiationexposed human populations, the LSS cohort has played a critical role in obtaining the basic coefficients of risk estimation. Also, the data obtained from the LSS cohort have enabled evaluation of the scientific validity of the linear, no-threshold (LNT) model. So far, the dose – response relationship supported the LNT model in principle; however, the dose – response relationship below 100 mGy tends to fluctuate, which limits statistical significance in the increase in the incidence of cancer at lower doses. While the LNT model has been used for evaluating the cancer risk from radiation exposure, even the most-celebrated epidemiological study could not

uncover the uncertainties of the radiation effects below 100 mSv, which requires understanding of the molecular mechanisms of radiation-induced carcinogenesis.

CHERNOBYL ACCIDENT AND CHILDHOOD THYROID CANCER: The accident at the Chernobyl nuclear power plant on 26 April 1986 released a large amount of radioactive materials that resulted in radiation exposure in the populations of the affected regions. In particular, fallout of radioactive iodines resulted in exposure of local residents through ingestion of contaminated foodstuffs and inhalation, which caused childhood thyroid cancer as one of the main health effects of the accident. Among children and adolescents under 18 years in 1986, 6848 cases of thyroid cancer were reported between 1991 and 2005. A large case – control study of Belarusian and Russian children showed a very strong dose – response relationship, and the risk appeared to increase linearly with doses up to 1.5 – 2 Gy, whereas a statistically significant increase in risk was not observed below 200 mGy. The estimated ERR of thyroid cancer among children younger than 15 years at the time of the accident was 5.6/Gy. A recent analysis of thyroid cancer prevalence in the Belarus cohort showed a linear dose – response below 5 Gy with an excess risk of 2.15/Gy. The result of an analysis in the Ukrainian cohort also reported a linear dose – response relationship below 5 Gy, and the ERR was 1.91/Gy. In both the cases, no statistically significant increase in risk was observed below 100 mGy. Several ecological studies have also been conducted, and one study in Belarus and Russia reported statistically significant elevation of thyroid cancer risk in the settlements with an average thyroid dose of 50 mGy. It has been applied for the projected dose that needs to provide iodine thyroid blocking in recent International Atomic Energy Agency (IAEA) publication.

THYROID CANCER RISK BY MEDICAL EXPOSURE: Although the association between thyroid cancer and medical exposure was implicated in the early 1950s, systemic epidemiological studies were limited during the 1980s. A pooled analysis of seven studies

with organ doses to individual subjects was conducted in 1995. It included five cohort studies (atomic bomb survivors, children treated for tinea capitis, children irradiated for enlarged tonsils and infants irradiated for an enlarged thymus gland) and two case – control studies (patients with cervical cancer and childhood cancer). To estimate a dose-dependent increase in the thyroid cancer risk for exposure before age 15, the data from five studies were pooled. A linear dose – response relationship was observed, and the ERR was estimated to be 7.7/Gy. An elevated risk of thyroid cancer was observed at doses as small as 100 mGy; however, it was no longer statistically significant below this level

3.5 Heredity

GENETICS AND HEREDITY IN CANCER

We may wonder why some individuals who live an apparently healthy lifestyle die with cancer whereas individuals who have poor dietary and personal habits do not. The answer to why some individuals get cancer and others do not appears to lie at least in part in genetic makeup. There are a number of autosomal dominant diseases that are known to contribute to cancer risk. For example, familial polyposis increases risk of colon cancer, and dysplastic nevi predisposes to melanoma. Both of these conditions run in families. There are also autosomal recessive diseases that increase risk of certain cancers. For example, albinism increases the incidence of squamous cell carcinoma of the skin. Persons with inherited xeroderma pigmentosum have a marked inability to repair damage to DNA caused by the sun and therefore have an increased susceptibility to skin cancer.

Malignant cells of many tumors have chromosomal defects. In some cases the defect is a translocation (movement of genetic material from one chromosome to another), which could serve to place an oncogene next to an activating DNA sequence. In some cases there is a deletion of a specific chromosome band, which could serve to eliminate a cancer suppressor gene. And in

trisomy, there may be an extra gene that leads to expression of some abnormality. Several cancers have been associated with chromosomal abnormalities. The Philadelphia chromosome, a translocation of the long arm of chromosome 22, is present in chronic myelogenous leukemia. Evidence from twins suggests that this is an acquired rather than an inherited abnormality. Down’s syndrome (trisomy 21) has been identified as a risk factor in leukemia, and familial renal cancer has been associated with deletion of chromosome 13. Thus, genetic makeup can be an important factor in cancer risk.

Also important in individual cancer risk is age. Although cancer can be seen at all ages, the risk of cancer increases with age. This escalating risk may be attributed to several factors including increased length of exposure to carcinogens with longevity, increased statistical probability (the longer we live the more opportunities we have to get cancer), and a declining immune system.

EVIDENCE FOR HERITABLE PREDISPOSITION IN ANIMALS

Although numerous animal strains with a predisposition to specific cancers are known, the direct introduction of a gene resulting in an inherited tendency is a recent phenomenon that more starkly demonstrates the relevance of heredity to malignancy. Two laboratories have recently demonstrated that an oncogene construct can be introduced into a strain of mice, be passed to subsequent generations, and expose both the animal founding the pedigree and subsequent litters to a greatly heightened likelihood of cancer. In both experiments, all or part of a normal mouse myc gene was coupled to other regulatory regions and injected into the male pronuclei of fertilized single-cell mouse eggs. The injected eggs were then transferred into pseudopregnant foster mothers and the offspring examined to determine if the constructed gene had been successfully passed. Stewart et al, using a hormonally inducible mouse mammary tumor promotor (MTV) linked to the myc gene, were able to establish distinct lines of MTV/myc transgenic mice. In one

strain, the founder mouse developed a mammary adenocarcinoma expressing RNA transcripts corresponding to the fusion gene, during one of her early pregnancies. All evaluable female progeny of the first generation who had inherited the MTV/myc gene, also developed mammary adenocarcinomas during early pregnancies. In a second strain, 16 of 35 transgenic mice (46% incidence) in a four-generation pedigree spontaneously developed a wide variety of tumors, ie, breast and testis cancer, mast cell tumors, and lymphocytic malignancies of both B and T cells, evenly distributed among both sexes. The authors noted that the incidence might be falsely low due to the early killing of some animals. Transmission was compatible with an autosomal dominant mode with reduced penetrance. Among several gene constructs of Adams et al, the heavy chain locus enhancer, linked to all or part of the myc gene, was the most tumorigenic in transgenic mice. The authors found that animals inheriting the construct were almost universally predisposed to lymphoma.

EVIDENCE FOR HERITABLE PREDISPOSITION IN HUMANS

Several conditions exhibiting a classical Mendelian pattern of inheritance have cancer either as a common accompaniment of the phenotype or the major phenotypic trait. X-linked recessive immunodeficiency syndromes predispose most commonly to lymphomas. Well-known autosomal recessive examples include Fanconi's anemia, ataxia-telangectasia, and xeroderma pigmentosum, all predisposing to a variety of malignancies. Unlike the predisposition with recessive conditions, the autosomal dominant disorders usually have the benign or malignant tumor as the identifying phenotypic characteristic. Examples include retinoblastoma, Wilms' tumor, neurofibromatosis, malignant melanoma, and familial polyposis coli (FPC). Widely dissimilar percentages of these individual tumor types have a hereditary origin. Furthermore, several known or suspected autosomal dominant disorders have aggregations of two or more malignancies. These syndromes,

which include the Cancer Family Syndrome (CFS) and the three multiple endocrine neoplasia syndromes, show that several different malignancies may segregate in a single family. Families of patients with multiple primary malignancies also appear to have a heritable predisposition to cancer. Moertel found a 26% increase in incidence of cancer among the firstdegree relatives (ie, parents, siblings, children) of such individuals compared with relatives of patients without cancer or with a single neoplasm only. Others have reported similar findings.

A far more common familial pattern of malignancy is one in which two or more family members are affected by the same cancer, although no pattern of gene segregation is obvious on examination of the pedigree. Whether the cause of these familial aggregates is purely genetic, purely environmental, or a combination of both is unknown, but the latter explanation is favored. Several genetic-environmental interactions are already known or suspected. There is direct evidence in animals and indirect evidence in humans that many hormones/chemicals thought to be carcinogens or promoting agents need to be activated by endogenous enzymes to have their ultimate deleterious effect. Individual variation in the activity of enzymes and in the metabolism of exogenous and endogenous agents is well described, and as these differences are likely to be genetically determined in many cases, differing susceptibilities of families to cancer on a hereditary basis would not be unexpected. Radiation also clearly interacts with a heritable predisposition to cancer.

Using the three leading causes of cancer mortality, breast, colon, and lung cancer, as examples, we will review the available evidence suggesting that a proportion of cancer incidence is influenced by heredity, and that genetic-environmental interactions are of importance. The review is meant to provide the reader with an overview of the several different types of evidence bearing on the question of heredity and cancer. Detailed reviews are available to the reader interested in the

entire spectrum of genetically transmitted disease associated with cancer development.

THE IDENTIFICATION OF SPECIFIC GENOMIC REGIONS INVOLVED IN HEREDITARY DISEASES

An examination of how specific genes or genomic regions directly involved in the genesis of neoplastic hereditary diseases have been identified, illustrates the power and promise that the study of heritable predisposition provides. The number of informative polymorphic markers available to study linkage with a disease trait has greatly increased due to molecular biologic techniques. By definition, informative polymorphic markers are heterozygous in the constitutional tissue of an individual; the specific chromosome containing a defined polymorphism can then be traced through related individuals and its segregation with a disease trait in a large pedigree, or a large number of families, can be statistically analyzed. The recent discovery of inherited polymorphisms in restriction endonuclease restriction sites in the genome has led to a seemingly unlimited increase in the number of polymorphic markers (called restriction fragment length polymorphisms [RFLPs]) available to investigators. As a result, much of the human genome is now accessible to study for its association to a disease trait. Clues from both clinical observations and cytogenetic abnormalities have provided genomic landmarks for linkage studies in hereditary neoplastic disorders. In retinoblastoma, rare individuals are found with constitutional (ie, all cells of the body are affected) deletions of the long arm of chromosome 13. These individuals, who often are also affected with various congenital abnormalities, have a retinoblastoma phenotype similar to that of patients with the hereditary form of the disease, that is, a higher incidence of bilateral tumors and younger age of onset than in the sporadic form of the disease. Subsequent cytogenetic analysis of the tumor tissue of hereditary retinoblastoma patients without a constitutional cytogenetic abnormality revealed similar deletions of chromosome 13, most commonly 13q14 suggesting that a gene important to retinoblastoma formation was located in this region.

This hypothesis was subsequently confirmed by the linkage of retinoblastoma with the esterase D gene and, later, RFLPs abutting the 13q14 region. In rapid succession, the putative gene has been isolated, characterized, and shown to be altered among retinoblastoma patients.
A comparable sequence of discoveries led to the identification of the genomic area involved in the development of Wilms' tumor. Rare patients have a constitutional deletion of the short arm of chromosome 11 and, as a result, develop a triad of aniridia, ambiguous genitalia, and mental retardation. These patients are also predisposed to develop Wilms' tumor. Subsequently, a similar deletion, specifically of 11pl3, was identified in the tumor cells of a patient without the triad, thereby implicating this part of the genome in Wilms' tumor carcinogenesis. The paucity of families with hereditary Wilms' tumor, due to the early age of death caused by this tumor until recent times, has not allowed linkage studies of families similar to those described for retinoblastoma; however, other evidence at the molecular genetic level implicates this genomic region in Wilms' tumorigenesis.

With the stimulus provided by the results in retinoblastoma and Wilms' tumor, the locations of genes for several additional disorders with increased susceptibility to cancer have been uncoveredfe. For these studies, investigators have employed linkage analyses conducted with RFLPs from several random chromosomes or, more selectively, from a chromosome often involved in a tumor's cytogenetic profile, when known.

3.6 Hormones

Role of hormones in endometrial cancer

The established risk factors for endometrial cancer show that exposure to estrogens unopposed to progestins can predict risk of endometrial cancer. During the premenopausal period, risk of endometrial cancer can be attributed to mitotic activity during the first half of the menstrual cycle when estrogen is unopposed

by progesterone. Use of sequential oral contraceptives (OCs) doubled the risk of endometrial cancer among women who used them prior to their removal from the market in 1976. In contrast, combination oral contraceptives (COCs), which deliver estrogen and a high dose progesterone for 21 days of a 28 day cycle, decrease the risk of endometrial cancer.

Obesity is also an important risk factor for endometrial cancer. In post-menopausal women, it is postulated that the conversion of androstenedione to estrone in adipose tissue results in the increased risk. In premenopausal women, obesity is thought to operate through increased anovulatory cycles and associated progesterone insufficiency. The protective effect of parity can also be explained by the unopposed estrogen hypothesis. The highest risk of endometrial cancer occurs in nulliparous women and risk decreases with each pregnancy. This is explained by the fact that no mitotic activity occurs during pregnancy due to the persistently high progesterone levels.

HORMONES AS CANCER GROWTH FACTORS

CELLULAR oncogenes and growth factors may be related in the sense that the products of some activated cellular oncogenes can be either growth factors or their specific receptors. Tumours that express both genes may then grow in an autocrine way. A growth model can be described in which some hormones take the role of "conventional" growth factors (eg, epidermal growth factors, platelet-derived growth factor). In such a model only the gene for the specific hormone receptor needs to be expressed for the cells to proliferate, making unnecessary the expression of the gene that, in the case of autonomous growth, governs the production of the growth factor itself. This model has some intriguing and interesting implications for cancers from both hormone-responsive and hormone-unresponsive organs.

CANCERS IN HORMONE-RESPONSIVE ORGANS

Oestrogen: The first implication is that in the case of a hormone that induces cell proliferation in an endocrine-related organ, such as oestrogens in breast tissue, the presence of the specific

receptor in cancer cells can be seen only as an advantage for the tumour itself; it will not have good prognostic significance in the absence of any hormonal manipulation. In breast cancer, it has been widely accepted that the presence of oestrogen receptors in some cells is due to the persistence of normal cell characteristics and must therefore be considered as a marker of differentiation. One of the implications of our model, however, is that the activation of the gene coding for the receptor confers an advantage on the cancer cells and is not a normal phenomenon. This view is in agreement with the fact, seldom mentioned or discussed, that oestrogen receptors are rarely expressed in normal adult breast cells and never in amounts such as those that may be reached in cancer cells. This fact leads us to propose that the oestrogen receptor, which is not expressed or is poorly expressed in normal circumstances except during puberty and pregnancy, may be re-expressed through the process of neoplastic transformation. Experiments to measure the degree of expression of this gene would probably show an amplification, correlated with the amounts of cellular oestrogen receptor, a phenomenon that could help in locating and identifying this gene.

One can take a step further. Tamoxifen, an oestrogen analogue that binds competitively to the specific receptor, can induce varying degrees of clinical response in patients with breast cancer, including long-term complete tumour regression.5 Since tamoxifen is devoid of cytotoxic effects, we can speculate that the tumour response may be due to clone extinction through differentiation. This hypothesis would imply that some cells in breast tumours depend for their continuous proliferation only on the presence of circulating oestrogen that binds to the receptor, whereas other cells may be totally autonomous, that is depending only on conventional growth factors. It is possible that the same cell may express receptors for oestrogen and be able to proliferate in an autocrine way. Experiments to assess whether or not this is possible would provide very useful information. A generalisation of these speculations leads one to ask whether a

cancer clone can stay in an undifferentiated state in the absence of growth factors. Growth factors may be absolutely needed to induce undifferentiated proliferation, and a cancer clone may be unable to maintain itself if growth factors and their receptors are not produced or are blocked. If this assumption proves to be valid, some interesting therapeutic strategies might be developed.

Progesterone: The role of progesterone in breast cancer remains unclear. There is a contradiction between the fact that in the developing breast progesterone acts in conjunction with rather than in opposition to oestrogens and reports showing a better prognosis - for breast tumours with progesterone receptors in the cancer cells. Use of anti progesterone agents in tumours known to contain progesterone receptors should help to solve this difficulty.

Prolactin: Prolactin can be more easily integrated into this theory of hormone-responsive cancer proliferation, since prolactin receptors can be found in malignant breast cells and since bromocriptine, an antiprolactin agent, has been reported to induce regression in some breast tumours. It may also be relevant that there is mutual reinforcement between oestrogen and prolactin and an inhibiting effect of glucocorticoids and testosterone on prolactin action; this fact could explain why chemotherapy associated with androgenic hormones or prednisone has been found to be more effective than chemotherapy alone If it could be confirmed that prolactin positively influences the proliferation of breast cancer, epidemiological investigations of the increasing use of prolactin inducers, such as tranquillisers, some antidepressant agents, hypnotic and antiemetic drugs often used in combination and for long periods, would become urgent. In addition, so that various types of chemotherapy and hormone therapy can be properly evaluated, it will become necessary to report whether or not such prolactin inducers were being administered at the same time.

CANCERS IN HORMONE-UNRELATED ORGANS

The concept of re-expression of receptors as part of the process of neoplastic transformation may explain the presence of hormone receptors, for example oestrogen receptors, in several endocrine-unrelated cancers. If genes for hormone receptors are unstable to the extent that the cancer process can re-express and amplify them long after they have played their part in normal circumstances, obviously they share several characteristics with the cellular oncogenes that have been discovered so far. Their presence in cancers of the kidney and the pancreas, for example, cannot be regarded as an oddity but as a normal feature in relation to cancer genetic instability. This does not mean, of course, that all cancers that bear hormone receptors are proliferating under the influence of the corresponding hormones, since these hormones may be absent due to age or physiological conditions. But some peptide factors such as somatomedin may be at work throughout life. One might take advantage of the interplay of hormones and receptors, for example by sequentially using tamoxifen and progesterone, if it is confirmed that tamoxifen binding to an oestrogen receptor may induce in turn a progesterone receptor, and that the binding of progesterone to its specific receptor exerts an inhibitory effect on cell proliferation. It seems relevant to search, in every case of cancer, for the receptors of every known hormone and to determine the extent to which cell proliferation is influenced by their presence, with the aim of using and even developing antagonising agents.

3.7 Autoimmune diseases

Autoimmune Diseases and cancer are diagnosed with increasing frequency, and there are many reports of an association between immune-mediated diseases and neoplasia. Various autoantibodies have been identified in both hematologic and epithelial malignancies. Paraneoplastic syndromes such as myasthenia gravis with thymoma, myasthenic syndrome

(EatonLambert) with lung cancer, and acanthosis nigricans with lymphoma and with adenocarcinoma are also recognized and are considered to be immune mediated. Furthermore, “rheumatic” manifestations such as carcinomatous polyarthritis are found in a variety of solid malignancies. On the other hand, other cancers, specifically lymphoreticular malignancies, are diagnosed with increasing frequency in many autoimmune conditions including the autoimmune rheumatic diseases. The attempt by numerous studies to estimate the risk of malignancy in various autoimmune diseases has been hampered by many methodological pitfalls.

MALIGNANCY AND VARIOUS AUTOIMMUNE DISEASES

Rheumatoid Arthritis: Many studies have investigated the association between rheumatoid arthritis (RA) and malignancy because of the high prevalence of this disease, which affects 1% to 3% of the general population. A cursory review of the literature suggests that the association between cancer in general and RA is equivocal. Proportional mortality studies have consistently shown a slight deficit of deaths from cancer in series of patients with RA. Cohort analyses of cancer morbidity, on the other hand, have shown an excess of observed number v expected number of cancers at all sites. However, the excess of cancer in patients with RA found in cancer morbidity studies was not, statistically significant in every instance. The apparent disagreement between the proportional mortality studies and the morbidity studies results from methodological errors that will be discussed later and from increased mortality from infections and respiratory, renal, and cardiovascular diseases in RA.

Special attention has been paid to the simultaneous occurrence of lymphoproliferative (LPN) neoplasms and RA. Recent, well controlled, retrospective and prospective studies have shown an increased incidence of reticuloendothelial cancers, especially lymphomas, Hodgkin’s disease, multiple myeloma, and leukemia. The largest study was conducted by Isomaki et al In this retrospective study, the incidence of malignant neoplasms among 46,101 individuals with RA in Finland collected from the Social Insurance Institution’s Population Register was

determined. The follow up comprised a total of 213,911 person-years. The relative risk for lymphoma in RA patients was 2.7 compared with the general population. Prior et al published a similar study on a consecutive series of 489 patients with RA seen at a medical center in Birmingham, England. In this series, the relative risk for developing lymphoproliferative disorder was 15. An excess of deaths caused by lymphoproliferation was also found by Fries et al and Laakso et al who conducted well-controlled prospective mortality studies with mean follow ups of 12 and 10 years, respectively Histologically, a wide range of types of nonHodgkin’s lymphomas and Hodgkin’s disease were found. In a series of 13 patients who developed non-Hodgkin’s lymphoma, 12 belonged to the B cell series by morphologic criteria. The mean age of onset of RA was 49 years and the mean age of diagnosing LPN was 62 years. The mean interval between the two diseases was 13 years (range, 2 to 25 years). The relatively long period between the onset of RA and the occurrence of LPN may explain the failure of some mortality studies to detect an excess of such malignancies in RA.

An increased risk for LPN in RA raised the question of the role of cytotoxic and immunosuppressive therapy in inducing these malignancies. The 2.7-fold excess of LPN reported by Isomaki et al was determined in the absence of immunosuppressive drugs. Similarly, none of the patients who developed LPN in the Birmingham study had received cytotoxic drugs. On the other hand, an excess of non-Hodgkin’s lymphoma has been found in virtually all states of impaired immune function in humans that have been studied, including renal and cardiac transplant patients treated with cytotoxic immunosuppressive drugs, longterm renal dialysis patients, and patients with immunodeficiency disorders. Thus, it would be surprising if patients with RA treated with cytotoxic immunosuppressive drugs did not have an increased risk for developing LPN. A prospective study in the United Kingdom of 1,634 patients with different immune mediated diseases treated with immunosuppressive drugs reported a tenfold increase in non-

Hodgkin’s lymphoma, and a separate analysis of the 643 patients with RA found a 13-fold increase in non-Hodgkin’s lymphoma. Thus, patients with RA have two to three times greater risk for lymphoproliferative malignancy in the absence of immunosuppressive therapy, which is further increased after such treatment. A similar situation is observed in Sjiigren’s syndrome (SS) to be discussed later.

Systemic Lupus Erythematosus

Unlike RA, few publications have attempted to associate systemic lupus erythematosus (SLE) with malignancy. Two studies suggested an increased risk of malignancy, but these series were smal and not well controlled, and the patients selected did not reflect the natural history of the disease. Therefore, it cannot be concluded that malignancy and SLE are related. SLE has been associated with lymphomas, but there are no good controlled studies supporting this notion. The belief that patients with SLE are at increased risk of developing lymphoma stems from animal models of SLE-NZB (NZB/ NZW) Fl and MRL/lpr mice-that spontaneously develop malignant lymphoma and monoclonal macroglobulinemia, and from case reports of patients with SLE associated with lymphoma. Green et al, in their report, describe several cases of lymphoma with SLE other authors referred to about 100 case reports of SLE associated with lymphoma. Another recent report reviews eight cases of Hodgkin’s disease that developed in patients with SLE. In 12 of the 18 patients described by Green et al.

the autoimmune disease preceded the appearante of the lymphoma. In these cases, the lymphoma was diagnosed at a mean age of 46 years, while SLE preceded the development of lymphoma by 2 months to 12 years. In four cases, the lymphoma was diagnosed as Hodgkin’s disease and in eight it was classified as non-Hodgkin’s lymphoma. Most non-Hodgkin’s lymphomas are thought to be of the B cell origin. Only three of the patients had been treated with immunosuppressive or cytotoxic drugs.

Chronic Lymphocytic Thyroiditis
There is a controversy regarding the risk of LPN in patients with chronic lymphocytic thyroiditis. Several studies have observed an increased risk of thyroid lymphoma in patients with thyroiditis. In a recent controlled study analyzing the incidence of malignant tumors and leukemia among 829 patients with chronic lymphocytic thyroiditis and 229 age- and sexmatched patients with colloid goiter, an increased risk of malignant thyroid lymphoma among the patients with thyroiditis was found (relative risk, 67). In this study patients with thyroiditis also had an increased risk of myeloproliferative and lymphoproliferative neoplasms, a situation resembling the extraglandular lymphoproliferation found in patients with SS(Sjiigren’s Syndrome).

Mixed Connective Tissue Disease Since mixed connective tissue disease (MCTD) includes the clinical features of PM, SLE, and/or systemic sclerosis, it might be anticipated that patients with this disease would show an increased incidence of cancer. However, MCTD as originally defined, ie, a relatively fixed benign disorder associated with a high titer of anti-RNP antibodies, is no longer accepted. Some patients go on to develop SLE, some develop scleroderma, and many develop major organ involvement. Only one study of the cancer rate among patients with MCTD has been reported. Forty patients with MCTD were followed for 96 patient-years. In 11 men, there were three cases of cancer (2 lung, 1 lymphoma), compared with an expected incidence of 0.13 (relative risk, 23; $P < .01$). In women there was a single ovarian cancer. The relative risk for cancer in the total group was 12.9 ($P < .01$).

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Chapter 4: Classification

Carcinoma

Carcinoma refers to a malignant neoplasm of epithelial origin or cancer of the internal or external lining of the body. Carcinomas, malignancies of epithelial tissue, account for 80 to 90 percent of all cancer cases.

Epithelial tissue is found throughout the body. It is present in the skin, as well as the covering and lining of organs and internal passageways, such as the gastrointestinal tract.

Carcinomas are divided into two major subtypes: adenocarcinoma, which develops in an organ or gland,

and squamous cell carcinoma, which originates in the squamous epithelium.

Adenocarcinomas generally occur in mucus membranes and are first seen as a thickened plaque-like white mucosa. They often spread easily through the soft tissue where they occur. Squamous cell carcinomas occur in many areas of the body.

Most carcinomas affect organs or glands capable of secretion, such as the breasts, which produce milk, or the lungs, which secrete mucus, or colon or prostate or bladder.

sarcoma

The term sarcoma refers to a tumor of connective tissue and derives from the Greek sarkos (flesh) and sarkoma (fleshy substance). Sarcomas comprise a heterogeneous group of mesenchymal neoplasms, including more than 100 distinct diagnostic entities. This heterogeneity can be identified by light microscopy and analyses of gene expression. Marked heterogeneity in biological behavior may exist even within a single histologic category. Because of the large number of subtypes of sarcoma, only the most common or instructive types will be discussed herein. Sarcomas can be grouped into 2 general types, soft tissue sarcoma (STS) and primary bone sarcoma, each of which has different staging and treatment approaches. Soft tissue sarcomas are typically classified on the basis of genetic alterations and light-microscopic examination of hematoxylin-eosin–stained tissue, in which recognizable morphological characteristics of normal tissues are identified. Sarcomas are further characterized by histologic grade. The 3 most important prognostic variables are grade, size, and location of the primary tumor.9 The symptoms that call attention to a sarcoma are usually those caused by its presence and growth at its site of origin. If the tumor originates in an easily visible site, the patient may present with an asymptomatic mass. If the tumor distorts normal structures, pain may be the presenting symptom. Therefore, retroperitoneal sarcomas often are large before they are brought to medical attention. In rare instances, paraneoplastic symptoms such as fever may occur. A biopsy (either open or large-gauge core needle) is needed to obtain adequate tissue for diagnosis of sarcoma. Care should be taken to ensure that the biopsy does not interfere with subsequent optimal definitive surgery. Because sarcomas are relatively uncommon, yet comprise a wide variety of different entities, patients should be evaluated by oncologists who have expertise in the field of sarcoma.

Blastoma

All cells have a life cycle. They exist for a certain amount of time, and then they die. The body's cells are constantly renewing themselves.

Cancer cells are ones that do not die at the natural time in their life cycle. Instead, they grow uncontrollably, spreading and causing tissue damage.

Blastoma is cancer that affects a type of stem cell known as a precursor cell in a fetus. A precursor cell is one that can become any type of body cell.

A developing baby that is not yet born has more precursor cells than an adult because the body is still forming. For this reason, blastoma is most common in children.

Lymphoma and leukemia

Pediatric lymphoma, including Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL), is the third most common malignant neoplasm in childhood and adolescence. HL is unusual in patients younger than 4 years of age, and typically occurs in older children and adolescents.3 In patients younger than 10 years of age, there is a male predominance of HL; beyond this age the relative incidence begins to equilibrate between the genders and patients older than 15 years of age have an approximately equal incidence between boys and girls. There are some studies suggesting an association between chronic Epstein-Barr virus (EBV) and HL and this has led some investigators to propose a distinction between the childhood and adolescent/young adult forms of HL. HL is characterized by a variable number of characteristic clonal multinucleated giant cells (ReidSternberg [RS] cells) in an inflammatory milieu. The WHO classification separates the uncommon nodular lymphocyte-predominant form of HL (NLPHL) from the common form, designated classic HL.WHO subtypes of classic HL are

nodular sclerosis (NS), lymphocyte rich (LR), mixed cellularity (MC), and lymphocyte depleted (LD). The NS subtype of HL accounts for greater than 85% of pediatric HL and is characterized by lymph nodes that have thickened capsules and dense collagenous bands that separate the nodes into macronodules. The presence of collagen and fibrous stroma contributes to the presence of residual mediastinal soft tissue that is commonly seen early after completion of therapy, even after no viable disease remains. The MC subtype accounts for 30% of the cases in young children and can be confused for NHL. The characteristic RS cell in classic HL is believed to arise from preapoptotic germinal center B-cells that cannot synthesize immunoglobulin and show constitutive activation of the nuclear factor k-B pathway, conferring resistance to apoptotic stimuli. EBV is associated with 15% to 25% of HL in developed countries and up to 90% in developing countries, most commonly in younger patients with MC histology. Despite this, EBV serologic status does not seem to be a prognostic factor in pediatric patients with HL, in contrast with patients with NHL.

Leukemia is the most common childhood malignancy, accounting for one-quarter to one-third of childhood malignancy cases. Nearly all childhood leukemia cases are the acute form. Acute leukemia is classified by the morphology, immunophenotype, and cytogenetics of the leukemic cells into acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). ALL and AML account for three-quarters and one-fifth of childhood leukemia cases, respectively. Chronic myelogenous leukemia (CML) accounts for less than 5% of cases of childhood leukemia, whereas juvenile myelomonocytic leukemia (JMML), a myelodysplastic-myeloproliferative syndrome, accounts for less than 1% of cases of childhood leukemia.1 There is a sharp peak in ALL incidences among children 2 to 3 years of age, with evidence that ALL initiates in utero2 AML rates are highest in

the first 2 years of life, decline to a nadir at 6 years of age, and slowly increase during the adolescent years.

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Chapter 5: Treatment

CHEMOTHERAPY

Although the use of chemotherapy in exotic animal oncology is increasing, the information in literature is limited and mainly based on case study findings and extrapolation from the human literature and the treatment of dogs and cats. Dosing of antineoplastic drugs should be done carefully because of their narrow therapeutictoxic range. Therefore, it is of great importance to understand the basic mechanisms of action and potential toxicities before attempting to treat any animal species with chemotherapy . Because recent reviews have discussed the basic pharmacology of common chemotherapeutics, this point is not be belabored in this article.20 Currently, there is a growing interest in combining anticancer drugs aiming at maximizing efficacy while minimizing systemic toxicity through the delivery of lower drug doses. Combining 2 or more agents has a greater response than when used alone. The development of a multiagent chemotherapy protocol is based on selecting agents that act at different phases of the cell cycle , have independent mode of actions, have synergistic effects to overcome drug resistance, and vary in their toxicities. Chemotherapy drugs can be administered by multiple routes, including orally, subcutaneously, intralesionally, intravenously, and intraosseously . In particular, repeated intravenous administration might be difficult in many exotic animal species because of limited vascular access and because of the high vesicant and irritant properties of many chemotherapy drugs. In human medicine, there is a major trend in the development of oral chemotherapy, driven by pharmacoeconomic issues as well as patient convenience and quality of life. The availability of more oral chemotherapeutics will facilitate future usage of chemotherapy in exotic animals. Regardless of the route chosen, clinicians administering chemotherapy need to take appropriate precautions to protect themselves, patients, and owners from contact with potential toxic chemotherapeutics .20 Chemotherapeutics are commonly dosed based on the body surface area (BSA) of a patient instead of the bodyweight (BW),

because the BSA is considered a better indicator of the metabolic mass.77 Because knowledge in exotic animal cancer treatment is limited, the use of BSA to derive interspecies equivalents for therapeutic dosages is opening treatment perspectives. Misunderstood and misinterpreted use of BSA conversions, however, may have unfavorable consequences, including underdosing, leading to treatment failure, and overdosing, inducing unexpected severe or even deadly adverse effects. Therefore, dose extrapolation between different animal species should be based on more advanced allometric and physiologically based pharmacokinetic modeling.146 Recently, Antonissen and colleagues22 demonstrated by allometric scaling a clear correlation (R2 >0.97) between BW and the elimination half-life (T1/2el) of carboplatin in different avian species, expressed by the formula, T1/2el carboplatin 5 0.1147 (log value of BW)0.3046. T1/2el could also be scaled with an acceptable correlation (R2 5 0.83) in different mammalian species (rats, cats, dogs, and humans)147–150 and different avian species (budgerigar, pigeons, ducks, cockatoos, and chickens).22,137 Allometric scaling within 1 animal class is, however, preferred. Furthermore, carboplatin clearance is highly correlated with the animals' glomerular filtration rate. Taking into account the enormous differences in physiology between different exotic animal species, interanimal class dose extrapolation of chemotherapeutics is related to a major risk of therapy failure.

Radiation therapy

Radiation is a physical agent, which is used to destroy cancer cells. The radiation used is called ionizing radiation because it

forms ions (electrically charged particles) and deposits energy in the cells of the tissues it passes through. This deposited energy can kill cancer cells or cause genetic changes resulting in cancer cell death. High-energy radiation damages genetic material (deoxyribonucleic acid, DNA) of cells and thus blocking their ability to divide and proliferate further . Although radiation damages both normal cells as well as cancer cells, the goal of radiation therapy is to maximize the radiation dose to abnormal cancer cells while minimizing exposure to normal cells, which is adjacent to cancer cells or in the path of radiation. Normal cells usually can repair themselves at a faster rate and retain its normal function status than the cancer cells. Cancer cells in general are not as efficient as normal cells in repairing the damage caused by radiation treatment resulting in differential cancer cell killing . Radiation can be given with the intent of cure as well as being used as a very effective modality of palliative treatment to relieve patients from symptoms caused by the cancer. Further indications of radiation therapy include combination strategies with other treatment modalities such as surgery, chemotherapy or immunotherapy. If used before surgery (neoadjuvant therapy), radiation will aim to shrink the tumor. If used after surgery (adjuvant therapy), radiation will destroy microscopic tumor cells that may have been left behind. It is well known that tumors differ in their sensitivity to radiation treatment. Table 1 shows a list of common cancers treated with radiation therapy. There are two ways to deliver radiation to the location of the cancer. External beam radiation is delivered from outside the body by aiming high-energy rays (photons, protons or particle radiation) to the location of the tumor. This is the most common approach in the clinical setting. Internal radiation or brachytherapy is delivered from inside the body by radioactive sources, sealed in catheters or seeds directly into the tumor site. This is used particularly in the routine treatment of gynecological and prostate malignancies as well as in situations where retreatment is indicated, based on its short range effects.

Surgery

Several types of surgery are helpful to people with cancer. Some surgeries are used in combination with other types of treatment. Types of surgeries include:

Curative surgery
Curative surgery removes the cancerous tumor or growth from the body. Surgeons use curative surgery when the cancerous tumor is localized to a specific area of the body. This type of treatment is often considered the primary treatment. However, other types of cancer treatments, such as radiation, may be used before or after the surgery.

Preventive surgery
Preventive surgery is used to remove tissue that does not contain cancerous cells, but may develop into a malignant tumor. For example, polyps in the colon may be considered precancerous tissue and preventative surgery may be performed to remove them.

Diagnostic surgery
Diagnostic surgery helps to determine whether cells are cancerous. Diagnostic surgery is used to remove a tissue sample for testing and evaluation (in a laboratory by a pathologist). The tissue samples help to confirm a diagnosis, identify the type of cancer, or determine the stage of the cancer.

Staging surgery
Staging surgery works to uncover the extent of cancer, or the extent of the disease in the body. Laparoscopy (a viewing tube with a lens or camera is inserted through a small incision to examine the inside of the body and to remove tissue samples) is an example of a surgical staging procedure.

Debulking surgery
Debulking surgery removes a portion, though not all, of a cancerous tumor. It is used in certain situations when removing

an entire tumor may cause damage to an organ or the body. Other types of cancer treatment, such as chemotherapy and radiation, may be used after debulking surgery is performed.

Palliative surgery

Palliative surgery is used to treat cancer at advanced stages. It does not work to cure cancer, but to relieve discomfort or to correct other problems cancer or cancer treatment may have created.

Supportive surgery

Supportive surgery is similar to palliative surgery because it does not work to cure cancer. Instead, it helps other cancer treatments work effectively. An example of supportive surgery is the insertion of a catheter to help with chemotherapy.

Restorative surgery

Restorative surgery is sometimes used as a follow-up to curative or other surgeries to change or restore a person’s appearance or the function of a body part. For example, women with breast cancer sometimes need breast reconstruction surgery to restore the physical shape of the affected breast(s). Curative surgery for oral cancer can cause a change in the shape and appearance of a person’s mouth. Restorative surgery may be performed to address these effects.

Immunotherapy

Immunity, inflammation, and cancer In order to delineate the underlying basis for cancer immune evasion and to design effective immunotherapies, it is essential to understand how cancer interacts with the immune system. Accumulating evidence over the last decade from mouse models and human patients with cancer has demonstrated the importance of the

immune system in recognizing and eliminating transformed malignant cells. The immune system also plays a critical role in promoting tumor progression. This dual role by which the immune system can suppress and/or promote cancer growth is termed cancer immunoediting and consists of three phases: elimination, equilibrium, and escape . In this Review Series, Teng and colleagues review recent developments in cancer immunoediting, particularly the importance of CD8+ T cells in cancer immunoediting and more broadly in tumors with an adaptive immune resistance phenotype . They further describe the characteristics of an adaptive immune resistance tumor microenvironment that affect survival outcome, including immune contexture, immunoscore, and the presence of tertiary lymphoid structures. They also discuss the temporal occurrence of cancer immunoediting in metastases. By understanding immunoediting in the tumor microenvironment, key pathways that suppress endogenous antitumor responses can be targeted, leading to potential novel therapies for patients with cancer. Similar to the dual roles of T cells in cancer immunoediting, inflammation also has two roles in shaping cancer development. The Review by Shalapour and Karin discusses how tumorrelated chronic inflammation shapes local and systemic immunity to promote an immunosuppressive tumor microenvironment and tumor development, whereas acute inflammation can enhance antitumor immunity by promoting DC maturation and function as well as effector T cell priming . Further understanding of how inflammation influences cancer development and progression will lead to novel strategies that enhance antitumor immunity by targeting immunosuppressive chronic inflammation. Immunosuppressive myeloid cells in tumor microenvironment It has been well recognized that growing cancers contain tumor-infiltrating lymphocytes (TILs), which are ineffective at tumor elimination in vivo but can exert proliferation and effector function when removed from the immunosuppressive tumor microenvironment. This is because cancer cells have developed mechanisms to avoid recognition and elimination by the immune

system The major mechanisms by which tumors evade destruction by the immune system include downmodulation of components of antigen processing and presentation machinery; recruitment of suppressor immune cells, such as regulatory T cells, myeloidderived suppressor cells (MDSCs), and tumor-associated macrophages; production of soluble factors associated with immunosuppression, such as TGF-β and IL-10; and upregulation of ligands for coinhibitory receptors that downmodulate TIL activity, such as programmed death ligand-1 (PD-L1). Marvel and Gabrilovich review recent developments in the role of MDSCs in cancer . They explain that, in addition to immunosuppression, MDSCs can directly support tumor growth and metastasis. They also discuss the most pertinent issues of MDSC biology and highlight how these cells may be used both as prognostic factors and as therapeutic targets clinically. The Review by Ugel and colleagues covers tumor-associate myeloid cells, including MDSCs and tumor-associated macrophages .They explain how tumor cells reprogram myeloid cells to create an immunosuppressive environment as well as to drive tumor progression directly by promoting cancer stemness, angiogenesis, epithelial-to-mesenchymal transition, and metastasis. They also discuss the molecular pathways leading to the differentiation of tumor-programmed myeloid cells and their potential roles as prognostic/diagnostic biomarkers and therapeutic targets in the clinic. Immune checkpoint blockade therapy The elucidation of mechanisms underlying cancer immune regulation has been instrumental in the recent success of immune checkpoint therapy using antibodies that block CTLA-4 and PD-1 pathways to treat patients with cancer. CTLA-4 was discovered by Pierre Goldstein in 1987 . Later, several groups independently proved that CTLA-4 functions as an inhibitory receptor both in vitro and in knockout mice . These discoveries led to James Allison’s seminal work in 1996, demonstrating that CTLA-4 blockade erased tumors in mice , which provided a rationale for the subsequent clinical development of CTLA-4–targeting antibodies. In 2011, the US Food and Drug

Administration approved anti– CTLA-4 antibodies (ipilimumab) for use in treating melanoma, which marked the beginning of a new era for cancer immunotherapy. In this Review Series, Buchbinder and Hodi review the current state of anti–CTLA-4 therapy, including clinical efficacy, associated toxicities, and combinatorial strategies with radiation therapy, chemotherapy, and PD-1 blockade to increase the efficacy of anti–CTLA-4 therapy . The clinical development of PD-1 blockade was dependent on a sequence of basic science discoveries. PD-1 was originally cloned by Tasuku Honjo in 1992 . Almost 10 years later, the ligand for PD-1 (PD-L1) was found independently by two research groups led by Lieping Chen and Gordon Freeman . Chen went on to demonstrate that many human cancers upregulate PD-L1 and that blocking of the PD-L1/PD-1 interaction by antibodies leads to tumor regression in mice (18). These discoveries paved the way for the clinical success of PD-1 blockade in treating advanced solid tumors . The Review by Chen and Han focuses on the history and current developments of anti–PD-1 therapy . They also discuss basic characteris- tics of anti–PD-1 therapy and how this therapy is distinct from the anti–CTLA-4 approach. CAR T cell adoptive immunotherapy CAR technology was first reported in 1993 by Zelig Eshhar and colleagues, who transduced T cells with chimeric genes encoding single-chain antibodies linked to a transmembrane region and an intracellular domain encoding the signaling adaptor for the T cell receptor (23). It was demonstrated that CAR T cell therapy could redirect T cell killing to cells expressing the antibody’s cognate antigen. Later, it was shown that CD19 CAR-transduced human peripheral blood T lymphocytes could eradicate lymphoma and leukemia in immune-deficient mice . In 2010, a case report showed an encouraging result using CD19 CAR T cells for treating a patient with lymphoma . Since then, CAR T cell therapies have shown impressive clinical outcomes in treating patients with relapsed or refractory B cell malignancies, including acute and chronic lymphocytic leukemia . CAR T cells targeting solid tumors have also been tested but have only

yielded modest results so far. Here, using the CD19 paradigm, Sadelain provides a comprehensive review on the development of CAR technology, its clinical efficacy in treating B cell malignancies, and lessons learned from CD19 CAR therapy as well as potential CAR therapy-associated toxicities . Therapeutic cancer vaccines and tumor neoantigens Despite impressive clinical outcomes achieved with immune checkpoint blockade and CAR therapies, the overall results of therapeutic vaccination against established tumors remain suboptimal, as clinical benefit for patients with cancer was largely noted as prolonged survival. The Review by Melief and colleagues explains that the reasons for lack of cancer eradication are suboptimal vaccine design and the presence of an immunosuppressive tumor microenvironment . They further discuss how better results may be obtained by improvements in antigen choice and vaccine design as well as appropriate treatments that reverse immunosuppressive mechanisms, such as PD-1 blockade. Among antigen choices, neoantigens that arise as a consequence of tumor-specific mutations have been postulated to be of particular relevance to the control of cancer upon vaccination because T cells for these antigens are not deleted by central tolerance mechanisms . However, the identification of tumor neoantigens has historically been time consuming and labor intensive. The review by Gubin and colleagues discusses recent advances in next-generation sequencing and epitope prediction that make the rapid identification of tumor neoantigens possible .They also discuss the use of tumor neoantigens in personalizing cancer immunotherapies.

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Chapter 6: Vaccine

Cancer vaccines may target either the antibody (humoral) or T-cell arm of the immune system . Antibodies can detect only antigens on the surface of intact tumor cells, whereas T cells can detect any protein antigen made in the cell, because the Major Histocompatibility Complex (MHC) molecules (like HLA in humans) act as an internal surveillance mechanism or spy within the cell to detect fragments of all proteins made in the cell and

carry them to the surface where they can be seen by T cell receptors (TCRs) . Thus, most cancer vaccines attempt to induce cytotoxic T cells that can kill tumor cells. Here, we will give examples of both types of cancer vaccines that we have developed based on mouse model preclinical studies and translated to human clinical trials with promising early results. With the increased focus on immunotherapy due to the success of checkpoint inhibitors and adoptive T cell therapy, as well as with the licensing of the first human therapeutic cancer vaccine, Sipuleucel-T , there is a revived interest in cancer vaccines , especially to induce immune responses in otherwise less-immunogenic tumors. Such cancer vaccines may also be aided by combinations with checkpoint inhibitors, or inhibitors of other negative regulatory cells and molecules, to allow the resulting T cells to have their maximum effect . Because cancers that are detected clinically have already evaded potential immunosurveillance, their antigens may not be optimal immunogens. We developed an approach we call epitope enhancement to modify amino acid sequences of epitopes to make them more immunogenic . This involves identifying amino acid residues critical for binding of the peptide to the MHC molecule without affecting TCR recognition, using a combination of predictions of primary and secondary anchor residues and of empirical assays, as well as residues that interfere with binding, and then making substitutions to increase affinity of the peptide for the MHC molecule. Then, the modified sequences have to be tested for binding affinity, immunogenicity in HLA-transgenic mice, and most importantly, for the ability to induce T cells that recognize not only the enhanced sequence but also the wild-type natural sequence since that is the sequence in the virus or tumor cell. We applied this to both viral and tumor antigens. For the current study, we first mapped epitopes binding to HLA-A*0201, the most common human class I MHC molecule, in the sequence of TARP (T-cell receptor gamma chain alternative reading frame protein) originally discovered by Ira Pastan’s lab . This protein is encoded by a different reading frame from the TCR gamma chain

so the amino acid sequence is unrelated to T cell proteins. TARP was found to be expressed in about 95% of prostate cancers and about half of breast cancers, and at all stages and Gleason types of prostate cancer. We then modified the sequences to increase affinity for HLA-A*0201 and tested these in a binding assay and for immunogenicity in HLA-A2 transgenic mice . One TARP epitope, 27–35, was already high affinity and none of the modifications increased that affinity. However, TARP 29–37, an overlapping but independent epitope, bound only moderately well to HLA-A*0201 but could be improved by replacing the C-terminal residue with valine to make 29–37-9V. That enhanced peptide induced T cells reactive with the wild type TARP sequence, whereas some others, such as TARP-29–37-3A, did not . Finally, these were tested for induction of human T cells in vitro and found to induce human T cells that could kill human cancer cells (MCF-7) expressing both TARP and HLA-A*0201, and not ones with either alone . Based on these results, we initiated a phase I clinical trial(NCT00972309) to test safety and immunogenicity of these two peptides in HLA-A*0201+ prostate cancer patients. We divided the subjects into two arms, one receiving the peptides emulsified together with GMCSF in Montanide-ISA51 given subcutaneously, and one receiving the same peptides pulsed together with keyhole limpet hemocyanin (KLH) as a source of help onto autologous dendritic cells (DCs), given intradermally . Autologous DCs were prepared monocytes from an apheresis pack cultured for 4 days with GM-CSF and IL-4 and then matured with interferon-gamma and lipopolysaccharide. We elected to treat stage D0 prostate cancer, the stage in which primary tumor had been definitively eradicated with surgery or radiation, but now a rising PSA in the absence of radiographically evident metastases is a biochemical indication of micro-metastatic tumor recurrence. This has the advantage that the tumor burden is small, like an adjuvant setting, but there is a parameter (PSA) one can measure to monitor effects on tumor growth without waiting for tumor to be detectable radiographically. In this setting, it has been shown

that the rate of rise (slope or doubling time) of the PSA is a valid predictor of clinical outcome measured as time to radiographic progression or to death and response to therapy . The practical problem with the widely used doubling time is that if the rate of PSA rise goes to 0, a desirable outcome, the doubling time becomes infinite, not a useful number for statistics, and if the PSA starts actually decreasing, an even better outcome, the doubling time becomes negative, not a meaningful number . On the other hand, the slope, proportional to the reciprocal of the doubling time, is a continuous variable, whether positive, 0, or negative. Thus, we used the slope (log (PSA)) as our measure, compared to the patient's own pretreatment slope measured at ≥ 4 time points over > 3 months during the 12 months prior to vaccination. Patients were immunized 5 times at 3-week intervals from week 3-week 15, and PSA values followed for at least one year.

Slope (log(PSA)) was determined at each time point from week 3 to that time point, and compared with that person's pre-treatment slope to determine the change in slope. Because the two arms were not statistically different, we were advised to combine them to increase statistical power. Of 40 patients treated on both arms, 71.8% had a decreased slope (log(PSA)) at 24 weeks ($p = 0.0012$) and 74.2% had a decreased slope at 48 weeks ($p = 0.0004$) [38]. 15% of the patients actually developed a negative slope, that is a decreasing PSA. This decreased tumor growth rate was corroborated by an independent analysis of tumor growth rate constant obtained by fitting the PSA values to an exponential growth curve , which found that the tumor growth rate constant fell in half ($p = 0.003$). Thus, the vaccine appeared to slow tumor growth in nearly ¾ of patients . When T cell responses were measured as interferon-gamma ELISPOT responses to the two vaccine peptides and the wild-type version of 29–37, 77.5% of patients made a new response not present at baseline (considered positive if ≥3-fold over background at at least two time points and statistically significant), but the magnitude of response did not correlate with clinical response .

Thus, the vaccine was safe and immunogenic, and showed preliminary evidence of clinical benefit, but we did not have an immune correlate of clinical activity. We hypothesized that differences in T cell function or avidity might correlate better, but a mechanistic correlate has not yet been identified. We had found earlier that human T cells raised against the TARP peptides could kill human tumor cells expressing TARP and HLA-A*0201, indicating that the epitopes were endogenously processed and presented in the tumor cells , but T cells from TARP-immunized patients in this study have not yet been tested for lytic activity. Nevertheless, based on the phase I results, a randomized placebo-controlled phase II trial (NCT02362451) has been opened. This trial extends the vaccine peptides to cover all of the TARP sequence (so called multi-epitope TARP), in addition to the original 2 peptides, to avoid limitations to HLA-A*0201. The phase II trial is being conducted with autologous DCs because this arm showed a more significant change in slope on its own that the Montanide arm in the phase I study, and because making 7 emulsions would have been impractical. However, another parameter was discovered that might help explain which patients responded, at least in the arm that received autologous monocyte-derived DCs . It was found that a combination of genes that correlated with a tolerogenic DC phenotype was inversely associated with a greater decrease in PSA slope and with poor immunological response . By ROC curves, this gave 85% power to discriminate among PSA responders vs non-responders, and 98% power to discriminate among immunological responders and non-responders. Moreover, a simplified combination of 4 parameters, increased CD14 levels, increased IL-10 and CCL2 secretion, and decreased CCL22 secretion, correlated with similar predictive power . This ability of the DC properties to predict DC vaccine effectiveness has important implications for optimizing DC-based vaccines. We now turn to a vaccine that functions through an antibody response. In this case, it is fortunate that the driver oncogene product, HER2, is expressed on the surface of cancer cells where antibodies can detect it on

intact cells. One can think of it as a receptor that is constitutively driving the cells to proliferate. We know that antibodies to HER2 can be effective at least in some breast tumors, because trastuzumab and other anti-HER2 antibodies are approved for this use. However, no vaccine has been developed to induce a patient to make her/his own antibodies to HER2 that are effective. However, there are peptide-based and other vaccines under study to induce T cell responses to HER2 . For mouse preclinical studies, we made an adenovirus vector expressing the extracellular (EC) transmembrane (TM) and domains of rodent HER2 , and immunized HER2- transgenic mice and mice bearing large established TUBO tumors . The TUBO tumors derive from a BALB/c mouse transgenic for the rodent HER2 oncogene, and express high levels of HER2. We were gratified to see that even tumors 2 cm in diameter regressed completely within about 3 weeks after one dose of the vaccine . Large established lung metastases also completely regressed. Thus, this vaccine fulfilled the key requirement that it could treat large established tumors, not just prevent ones injected after the vaccination. We had intended to make a vaccine to induce a T cell response, but it turned out to our surprise that the mechanism was completely dependent on antibodies. CD8 T cells were not necessary, as they could be depleted prior to vaccination, and as beta-2 microglobulin knockout mice (without MHC class I molecules), which lack CD8 T cells, were protected as well as wild type mice. CD4 T cells could be depleted after the first 2 days, when they were needed to provide help for an antibody response, without affecting tumor rejection, so effector CD4 T cells were also not required. However, the vaccine did not work in JH knockout B-cell deficient mice. Also, serum from immunized mice could transfer the protection . Thus, the protection was purely antibody-mediated. However, unlike trastuzumab, which was shown to require Fc receptors [53], the protection was just as effective in FcR deficient mice. Moreover, serum from the immunized mice could kill a pure population of TUBO tumor cells in vitro (in the absence of cells that could mediate antibody-

dependent cellular cytotoxicity), and at 1:100 or 1:20 dilution, immune serum could inhibit phosphorylation of HER2 on the cell surface, suggesting that it worked by inhibiting oncogene function, not by cytotoxicity. Thus, the mechanism was different from that of trastuzumab, and the vaccine might work in patients who had failed trastuzumab. The vaccine also had the advantage that it did not require multiple expensive intravenous infusions of immunoglobulin every few weeks for the life of the patient. Polyclonal antibodies induced by the vaccine might also be more resistant to escape mutations than a monoclonal antibody. Based on these preclinical results, we translated this vaccine to humans, making a cGMP version of the adenovirus expressing the EC and TM domains of human HER2. By omitting the intracellular domain, we avoided any chance of oncogenicity or reversion to an oncogenic phenotype. To avoid neutralization of the adenovirus in adenovirus-seropositive people, we used the adenovirus to transduce autologous DCs as the vaccine, and showed that this approach also worked in mice .Patients with advanced HER2+ metastatic cancers who had failed all standard therapies available were immunized at 4–8 week intervals from week 0 to week 24 with escalating doses of autologous transduced DCs, and followed for 2 years after the last dose of vaccine for safety assessment. Because of the approximately 7% cardiotoxicity rate in patients receiving long-term trastuzumab therapy, we wanted to avoid testing safety of the adenovirus vaccine in patients previously exposed to trastuzumab or other HER2-directed therapy. Thus, part I of the trial (NCT01730118) was designed to treat patients naïve to these agents, mostly patients with non-breast tumors that expressed 1+ to 3+ levels of HER2 who were not eligible for trastuzumab. If safety was shown in these, then in Part II, we would proceed to treat breast cancer patients with 3+ levels of HER2 who had failed other HER2-directed therapies. Enrollment in Part I of the trial has been completed and we have seen no evidence of cardiotoxicity, despite frequent monitoring of left ventricular ejection fraction. At the lowest dose of 5 million autologous DCs, we saw no

clinical responses (and no antibody responses), but at 10 and 20 million DCs, 5/11 evaluable patients, with metastatic cancers that had failed all standard therapies, showed evidence of clinical benefit (either complete response, partial response, or stable disease lasting ≥ 6 months) (Wood et al, manuscript in preparation). Several patients have also shown significant decreases in the number of circulating tumor cells, often almost complete disappearance. Antibody responses for later dose groups are pending, as are T cell responses. Based on this evidence and the safety profile, we have received approval to extend the treatment to 40 million DCs and to start enrollment in Part II of the study involving treatment of breast and other cancer patients who have progressed on licensed HER2-targeted therapies. If these promising results are borne out, then the next step would be a phase II efficacy trial. In conclusion, we have translated two types of cancer vaccines from mice to human clinical trials. One vaccine is to induce T cells to a cell-internal prostate antigen, TARP, using an epitope-enhanced cancer vaccine and demonstrating the utility of the concept of epitope enhancement. This vaccine appears to slow tumor growth in nearly three quarters of stage D0 prostate cancer patients, and is now in phase II trials. The second vaccine is to induce antibodies to a cell-surface tumor antigen, HER2, that is a driver oncogene product accessible to antibodies. This vaccine has shown preliminary evidence of clinical benefit in patients with advanced metastatic HER2+ cancers that have failed all other therapies, including complete response, partial response and stable disease lasting ≥ 6 months, and decrease in circulating tumor cells. Both vaccines make use of autologous DCs, and we have seen that certain qualities of such DCs are critical for the success of such cancer vaccines. We conclude that both categories of cancer vaccines (targeting both arms of the adaptive immune system) can be translated from preclinical murine models to human clinical trials with promising early results.

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